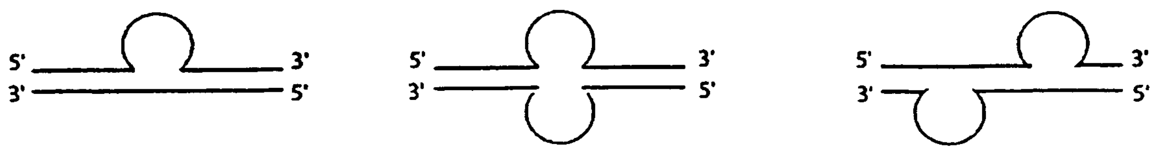
10/517638
Rec'd PCT/PTO PCT/US03/18499 13 DEC 2004

Figure 1



(a) (b) (c)

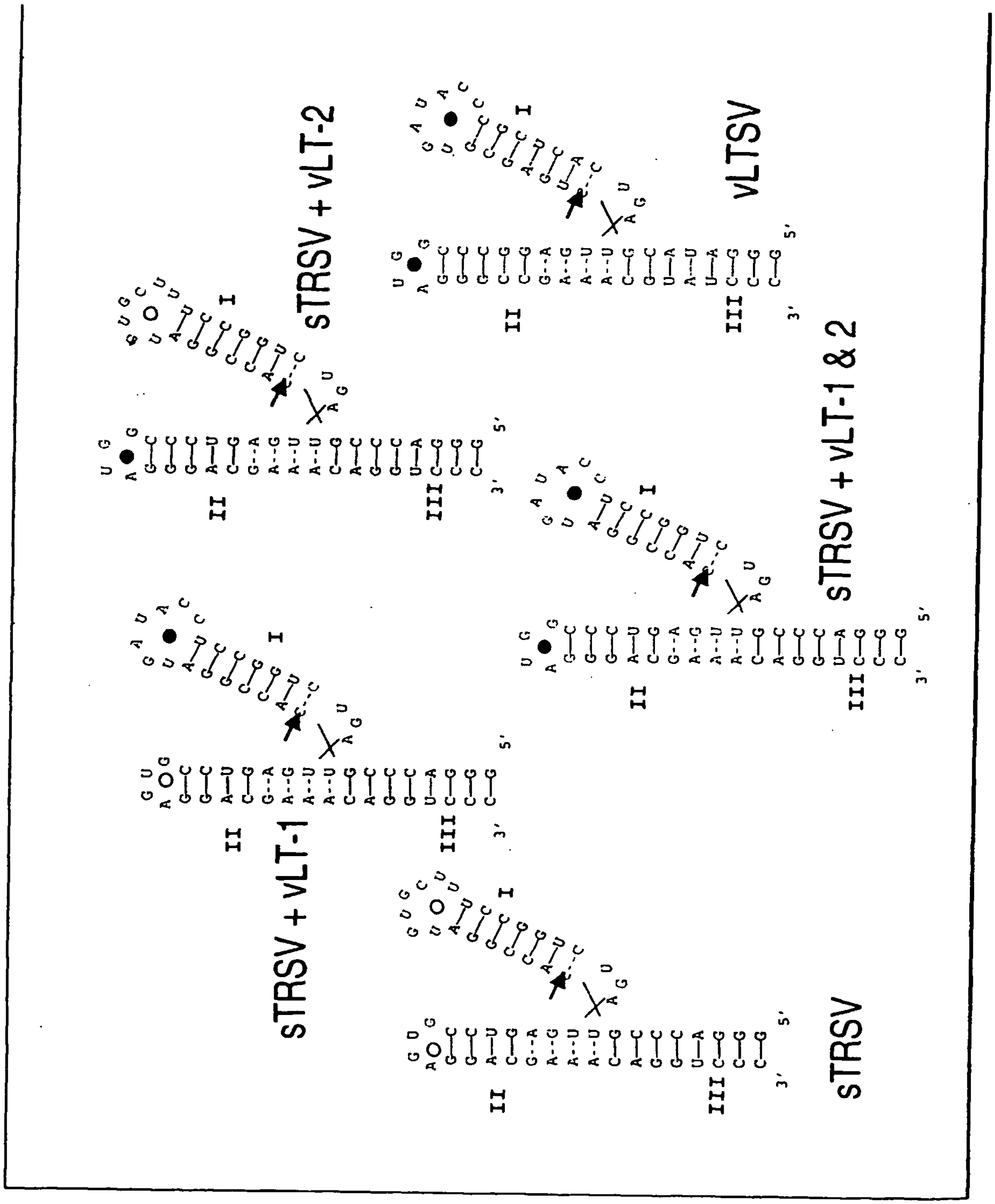


FIGURE 2

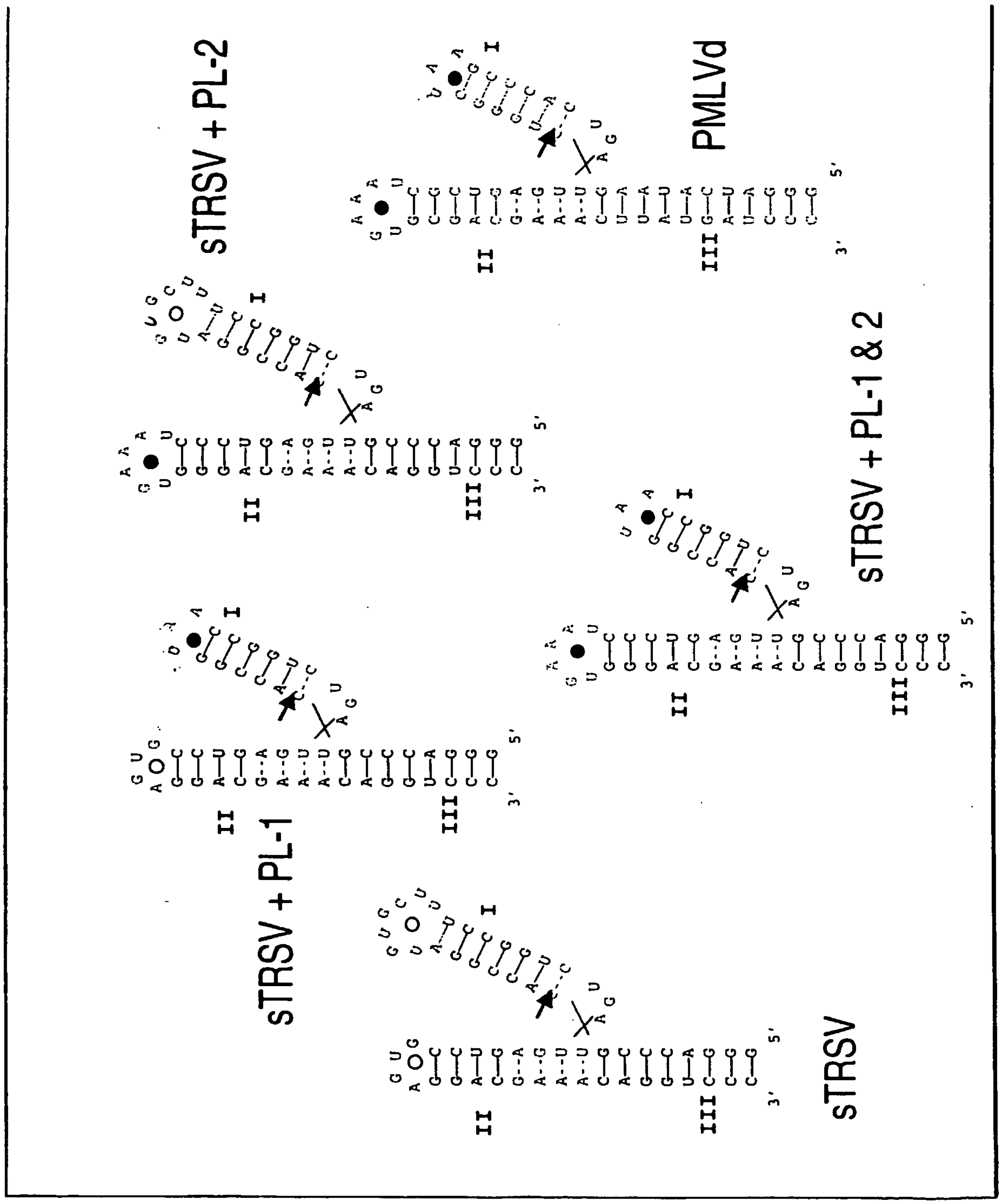


FIGURE 3

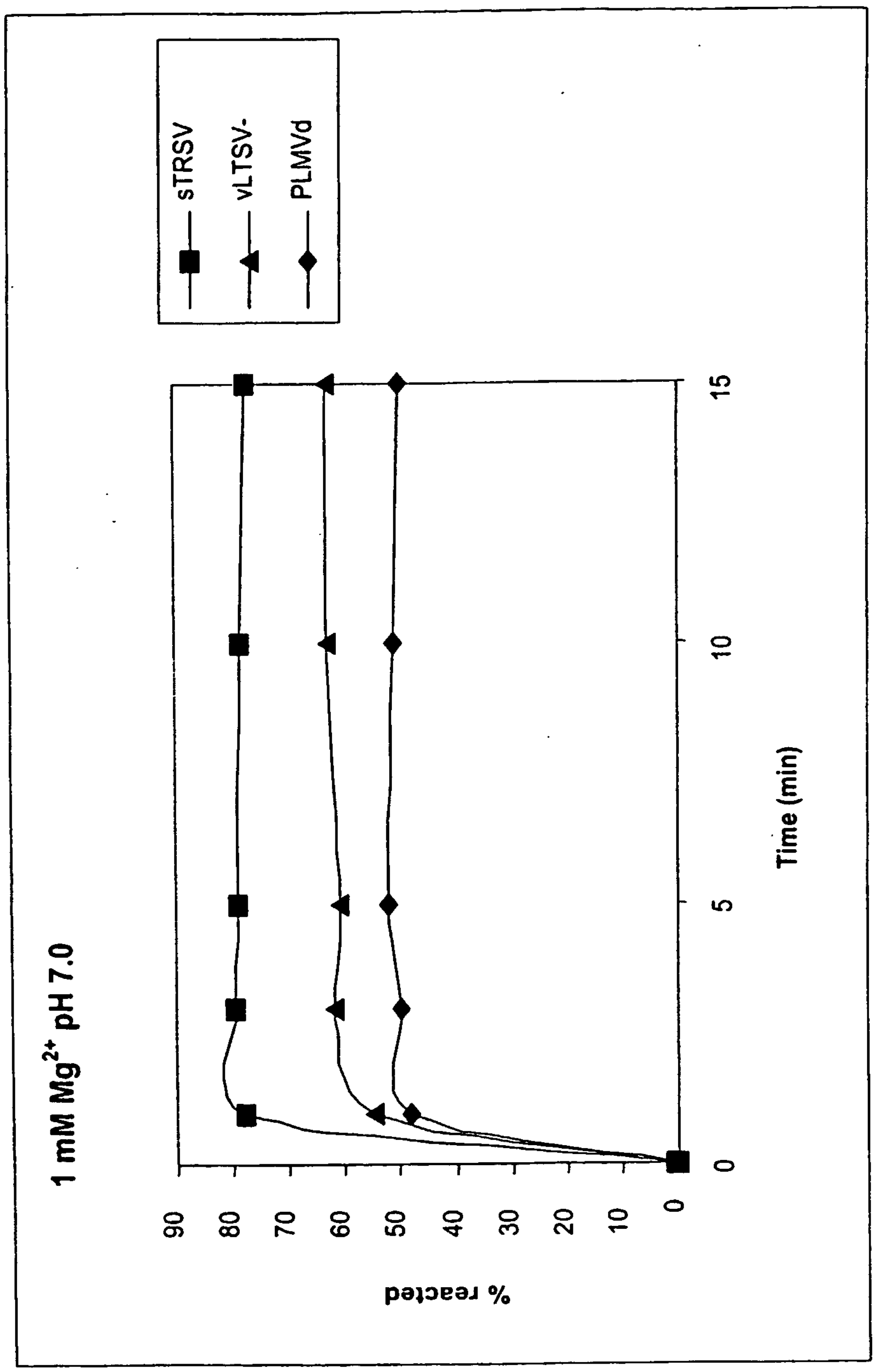

1 mM Mg2+ pH 7.0
% reacted
90
80
70
60
50
40
30
20
10
0
0
5
10
15
Time (min)
sTRSV
vLTSV-
PLMVd

FIGURE 4A

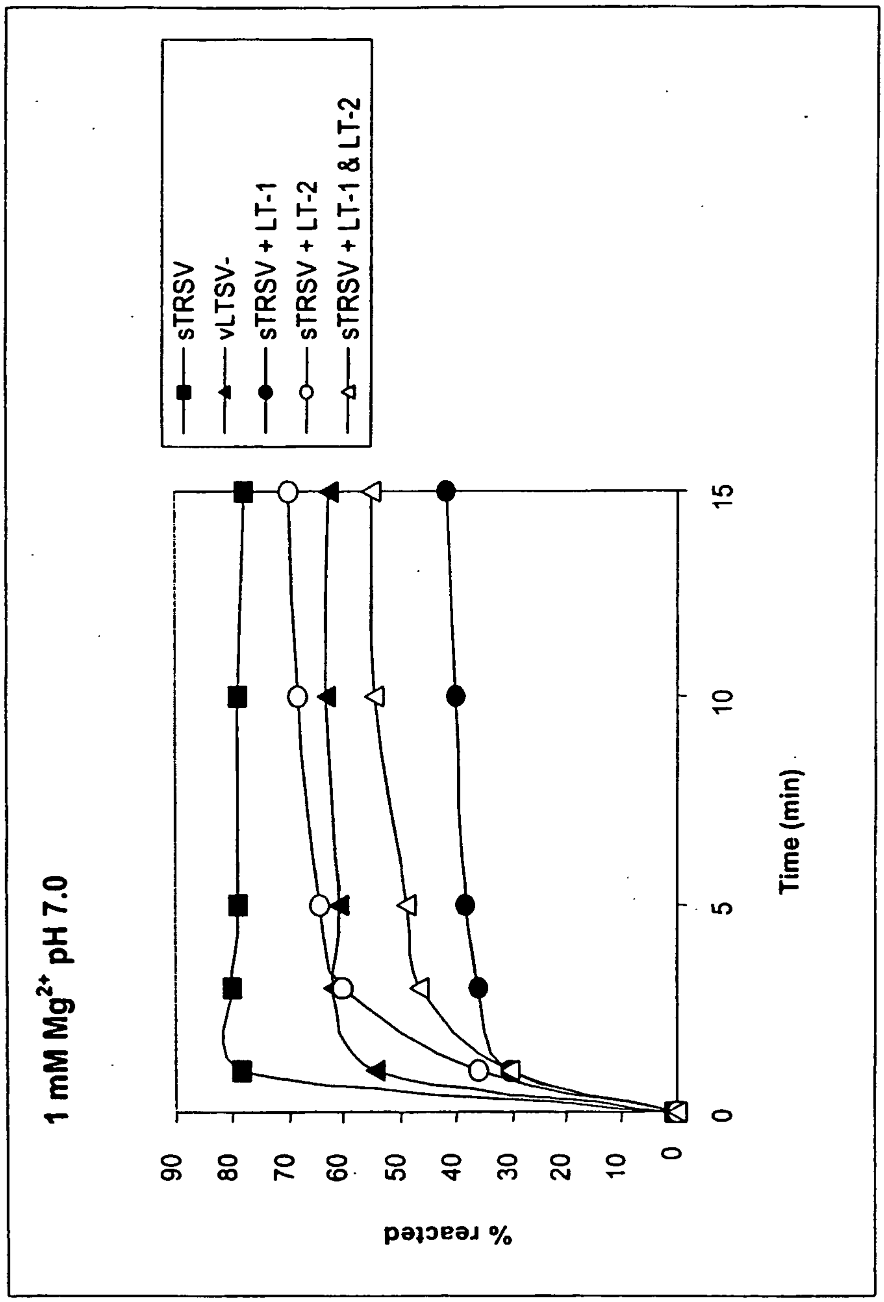

1 mM Mg2+ pH 7.0
% reacted
90
80
70
60
50
40
30
20
10
0
0
5
10
15
Time (min)
sTRSV
vLTSV-
sTRSV + LT-1
sTRSV + LT-2
sTRSV + LT-1 & LT-2

FIGURE 4B

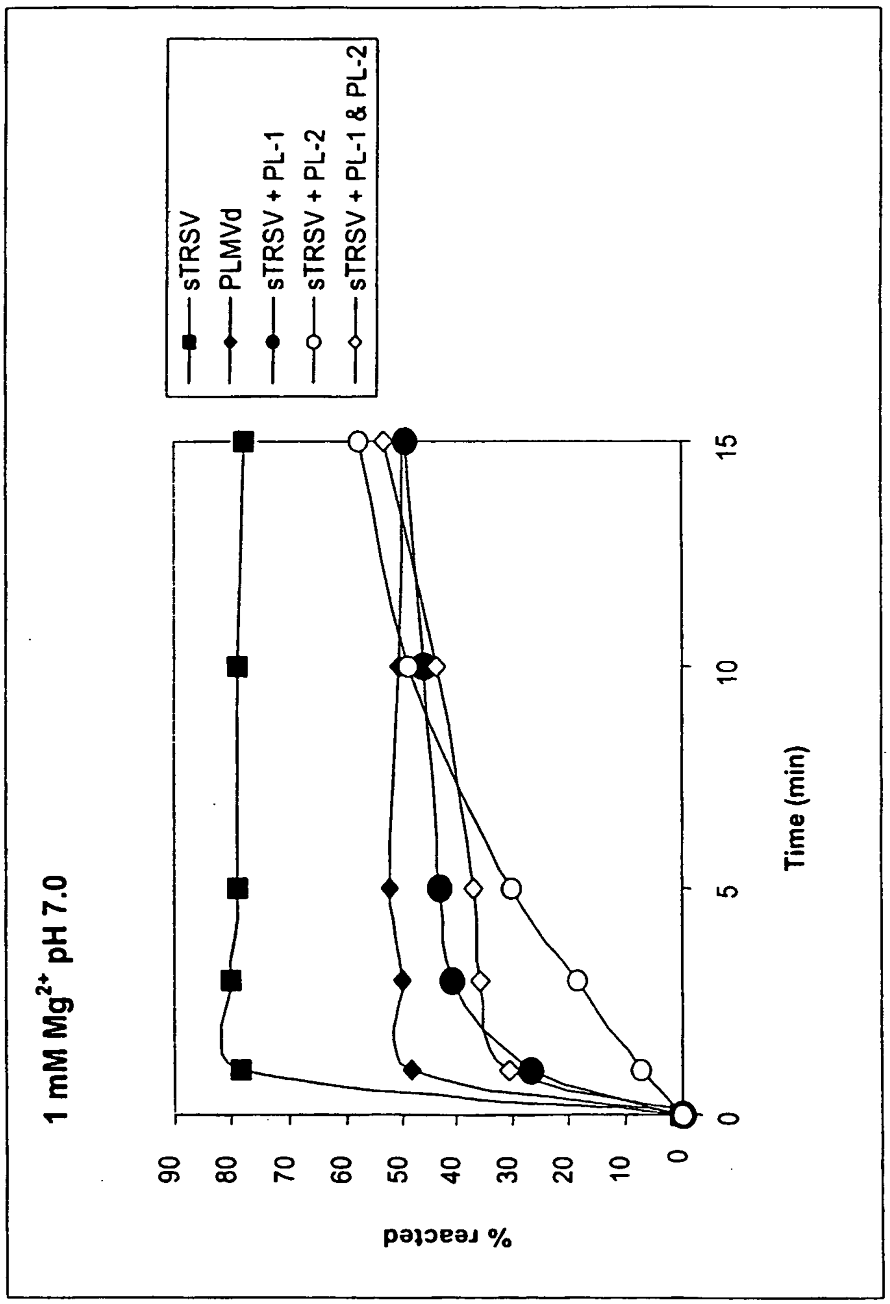

1 mM Mg2+ pH 7.0
% reacted
Time (min)
sTRSV
PLMVd
sTRSV + PL-1
sTRSV + PL-2
sTRSV + PL-1 & PL-2
0
10
20
30
40
50
60
70
80
90
0
5
10
15

FIGURE 4C

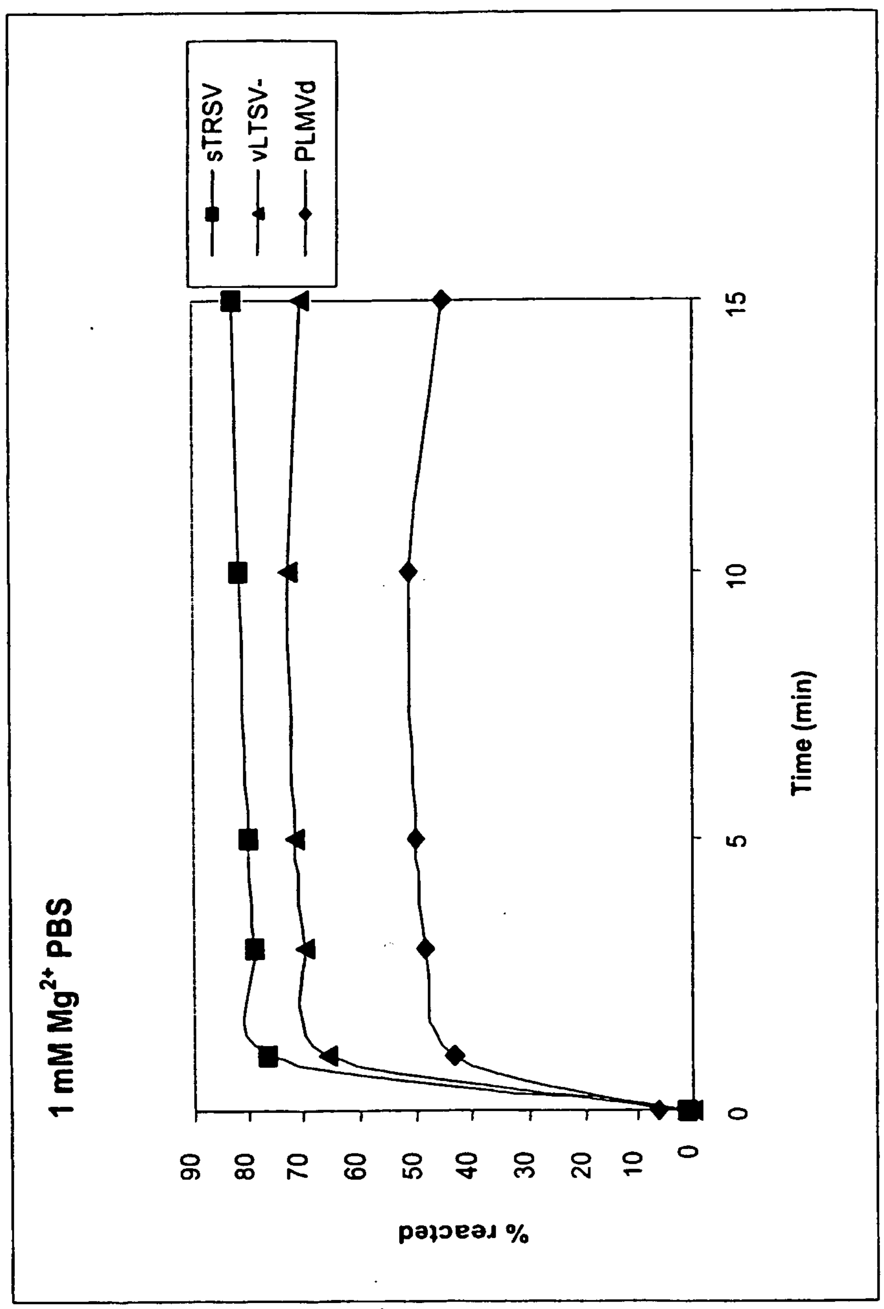

1 mM Mg2+ PBS
sTRSV
vLTSV-
PLMVd
% reacted
0
10
20
30
40
50
60
70
80
90
0
5
10
15
Time (min)

FIGURE 5A

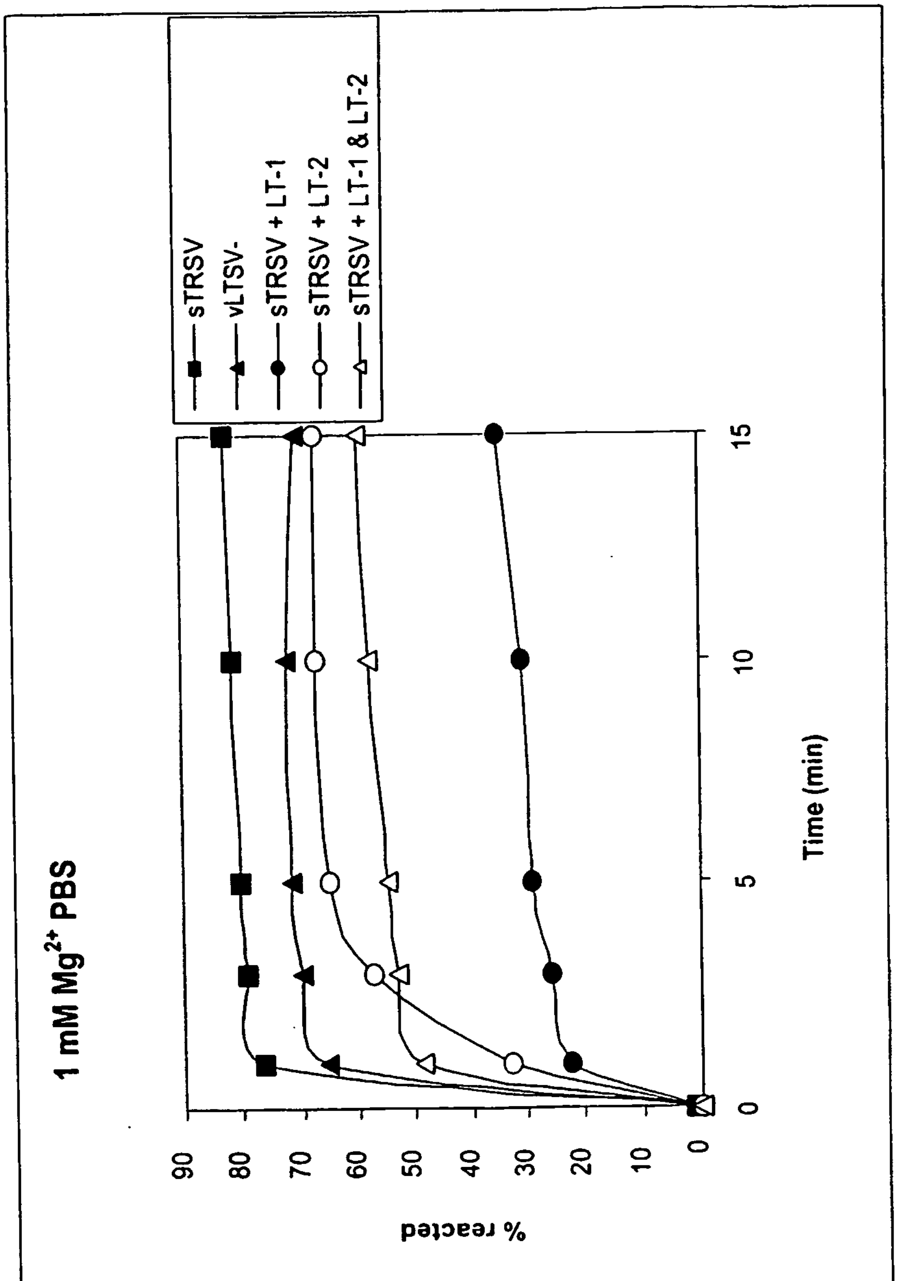

1 mM Mg2+ PBS
% reacted
90
80
70
60
50
40
30
20
10
0
0
5
10
15
Time (min)
sTRSV
vLTSV-
sTRSV + LT-1
sTRSV + LT-2
sTRSV + LT-1 & LT-2

FIGURE 5B

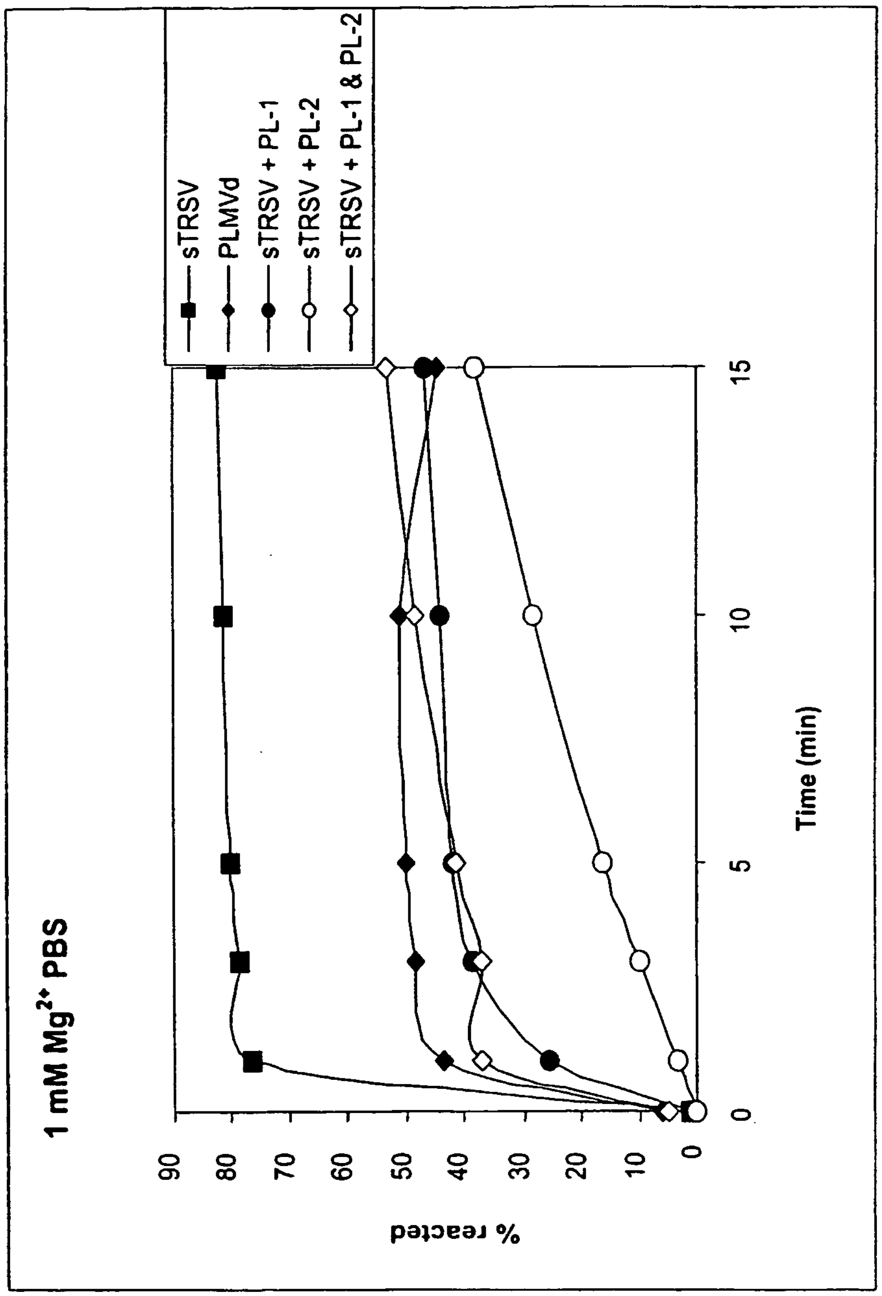

1 mM Mg2+ PBS
sTRSV
PLMVd
sTRSV + PL-1
sTRSV + PL-2
sTRSV + PL-1 & PL-2
% reacted
0
10
20
30
40
50
60
70
80
90
Time (min)
0
5
10
15

FIGURE 5C

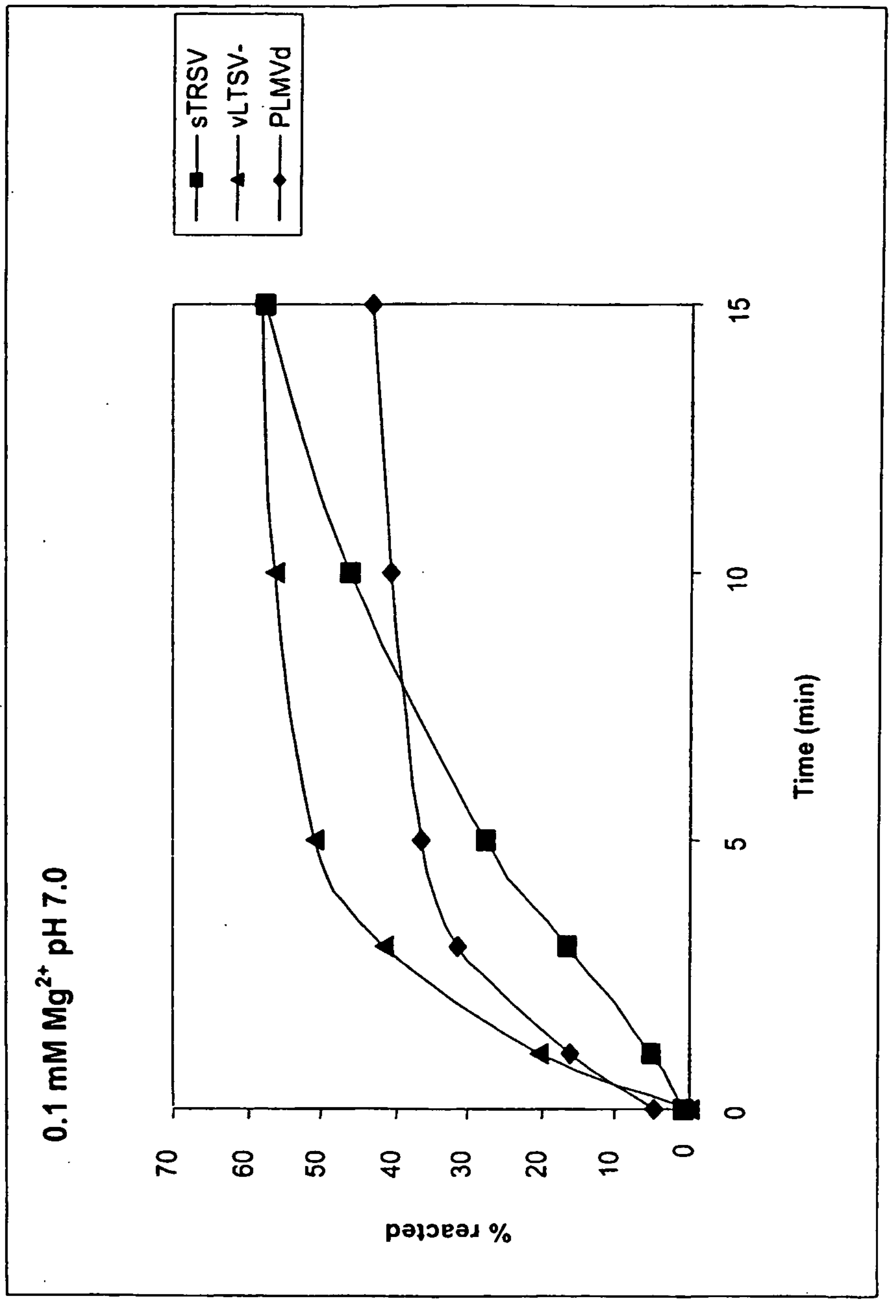

0.1 mM Mg^{2+} pH 7.0
% reacted
70
60
50
40
30
20
10
0
0
5
10
15
Time (min)
sTRSV
vLTSV-
PLMVd

FIGURE 6A

FIGURE 6B

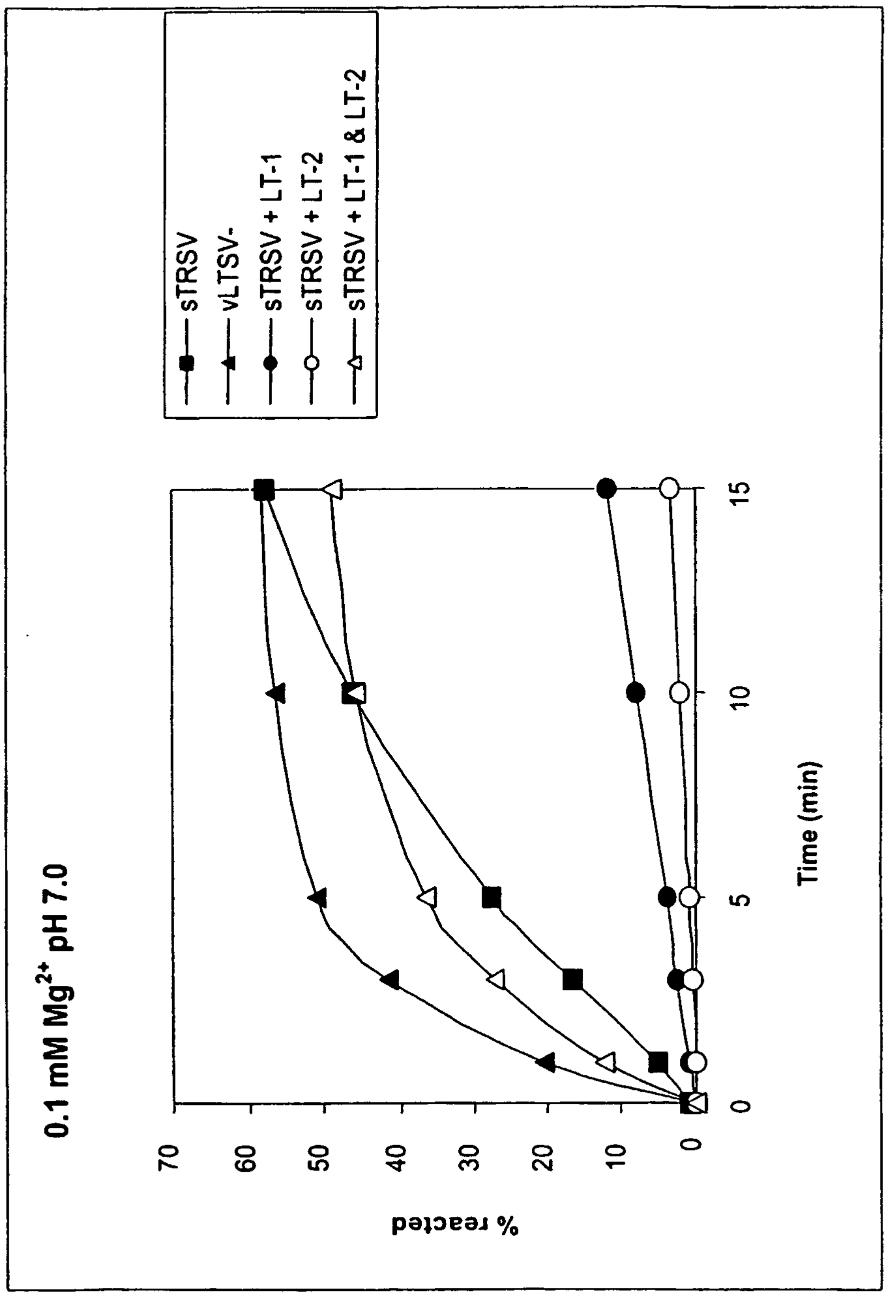

0.1 mM Mg^{2+} pH 7.0
% reacted
70
60
50
40
30
20
10
0
0
5
10
15
Time (min)
sTRSV
vLTSV-
sTRSV + LT-1
sTRSV + LT-2
sTRSV + LT-1 & LT-2

FIGURE 6C

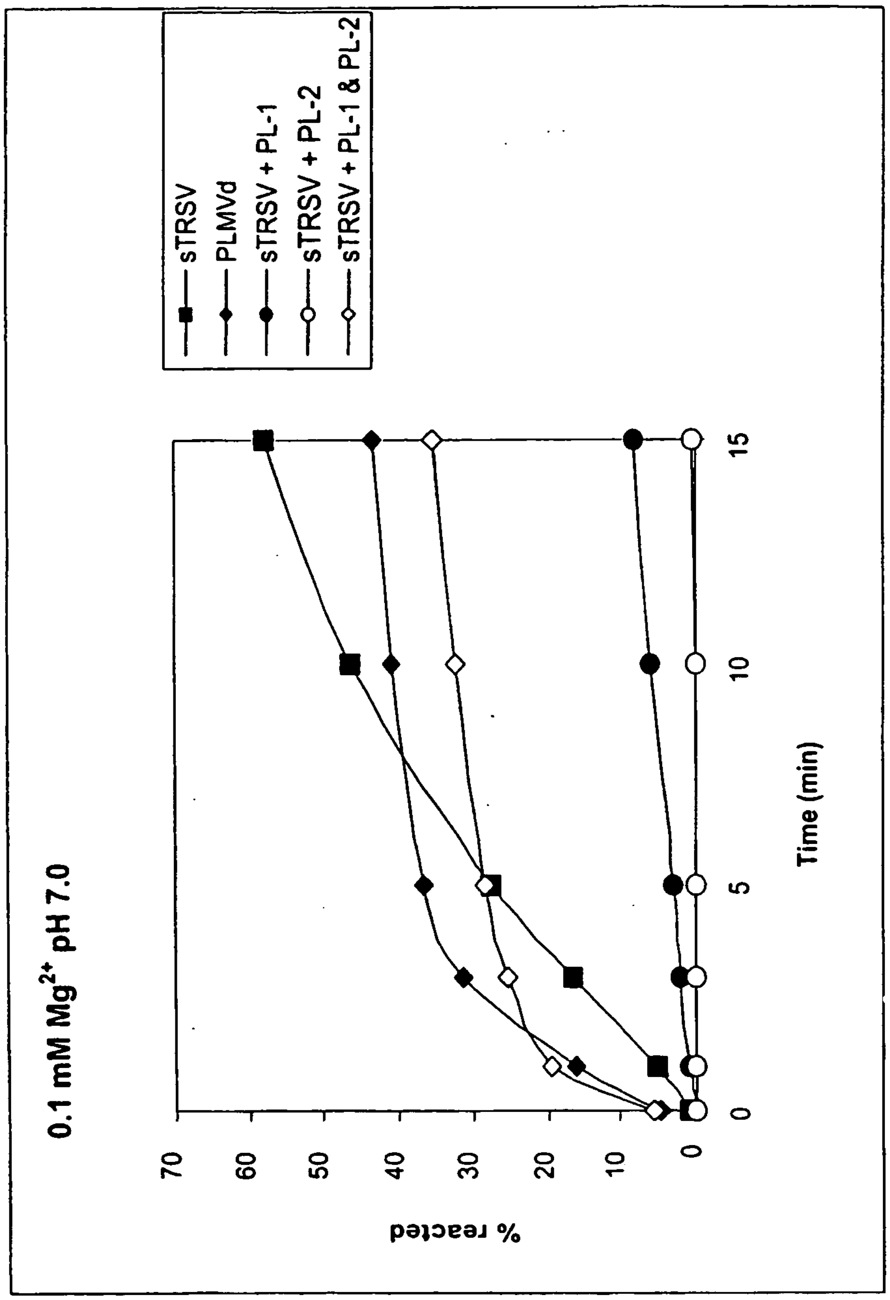
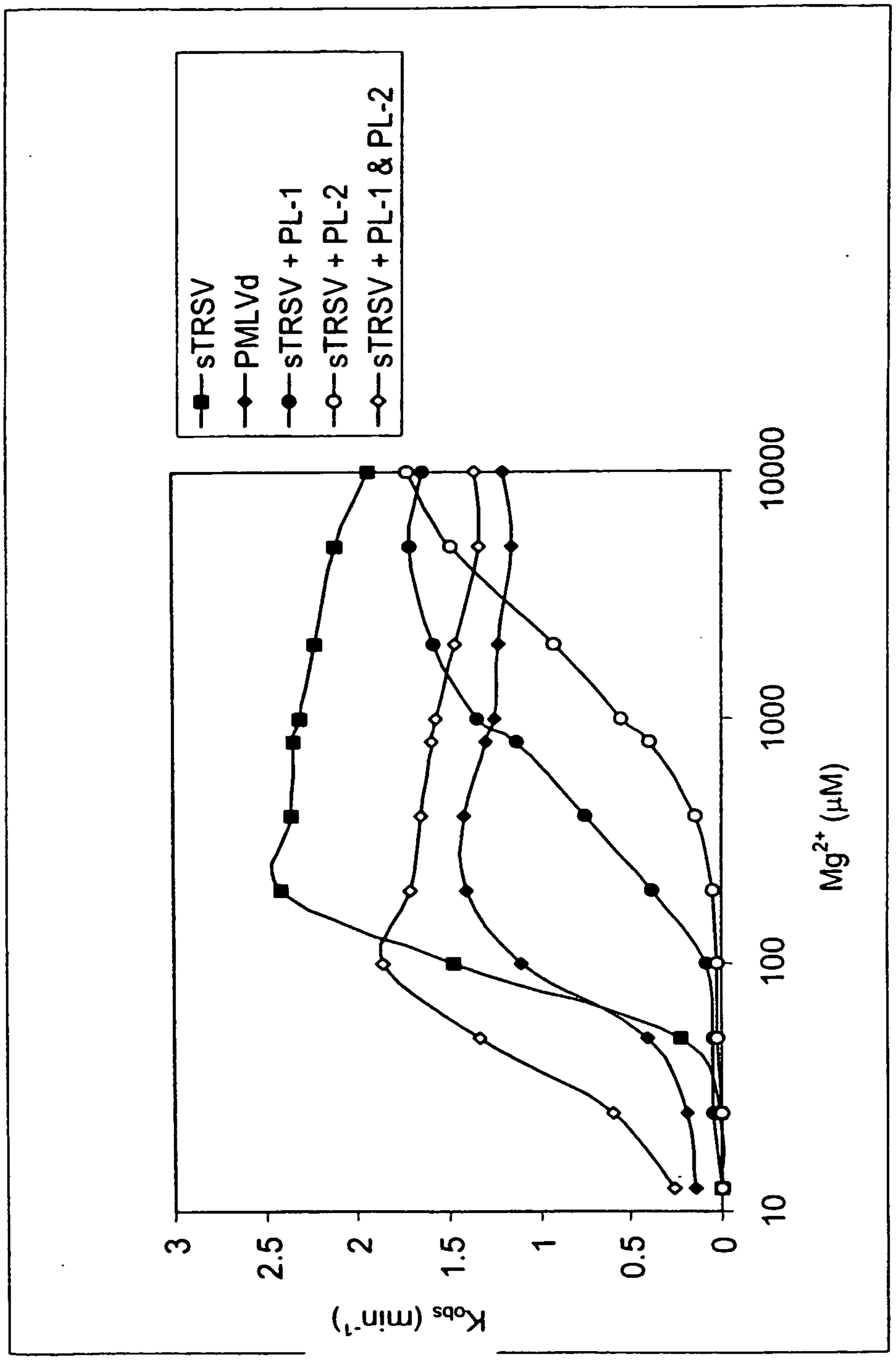

0.1 mM Mg2+ pH 7.0
% reacted
70
60
50
40
30
20
10
0
0
5
10
15
Time (min)
sTRSV
PLMVd
sTRSV + PL-1
sTRSV + PL-2
sTRSV + PL-1 & PL-2

FIGURE 7


sTRSV
PMLVd
sTRSV + PL-1
sTRSV + PL-2
sTRSV + PL-1 & PL-2
K_{obs} (min^{-1})
3
2.5
2
1.5
1
0.5
0
10
100
1000
10000
Mg^{2+} (μM)

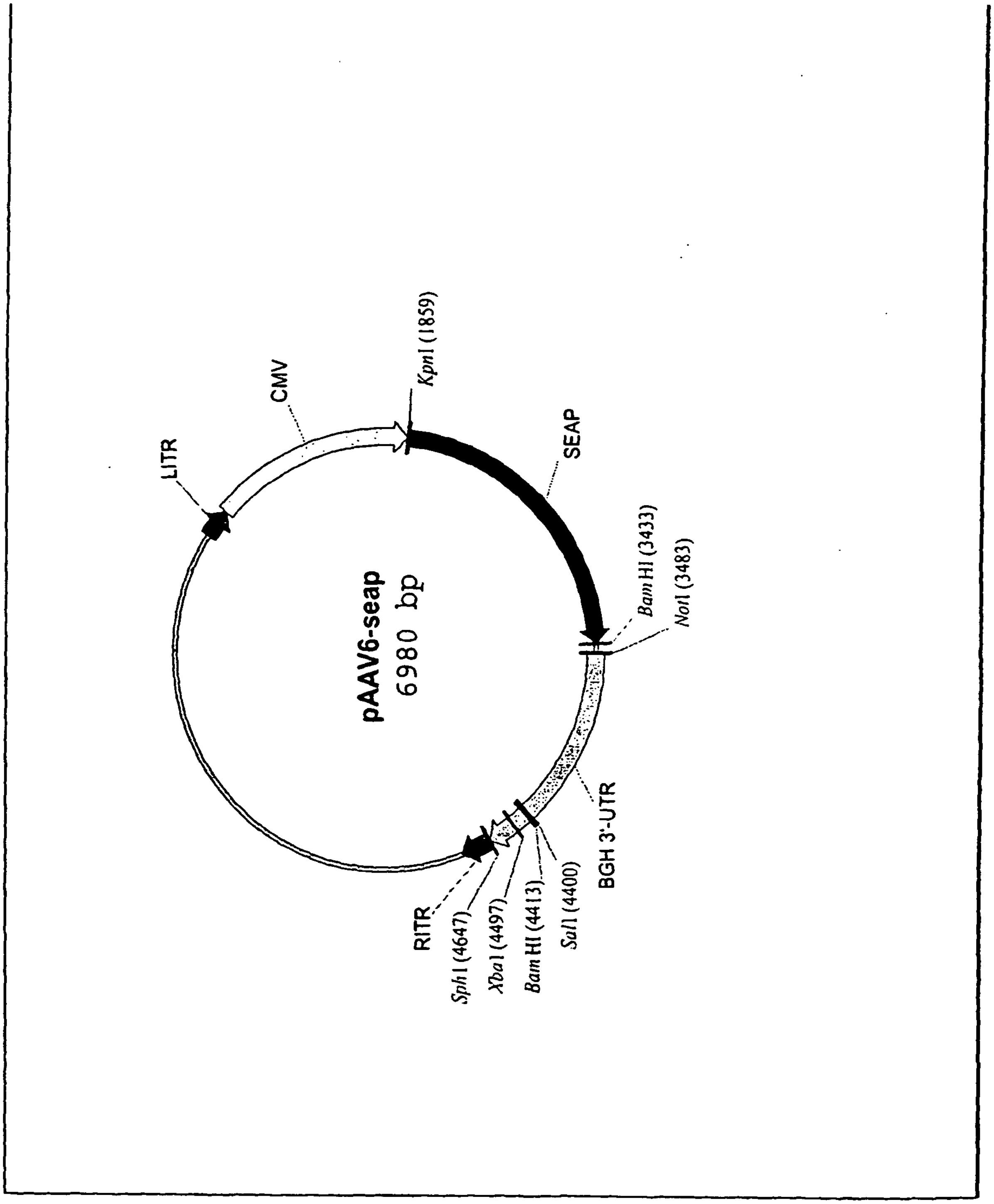

LITR
CMV
KpnI (1859)
SEAP
BamHI (3433)
NotI (3483)
pAAV6-seap
6980 bp
BGH 3'-UTR
SalI (4400)
BamHI (4413)
XbaI (4497)
SphI (4647)
RITR

FIGURE 8

FIGURE 9

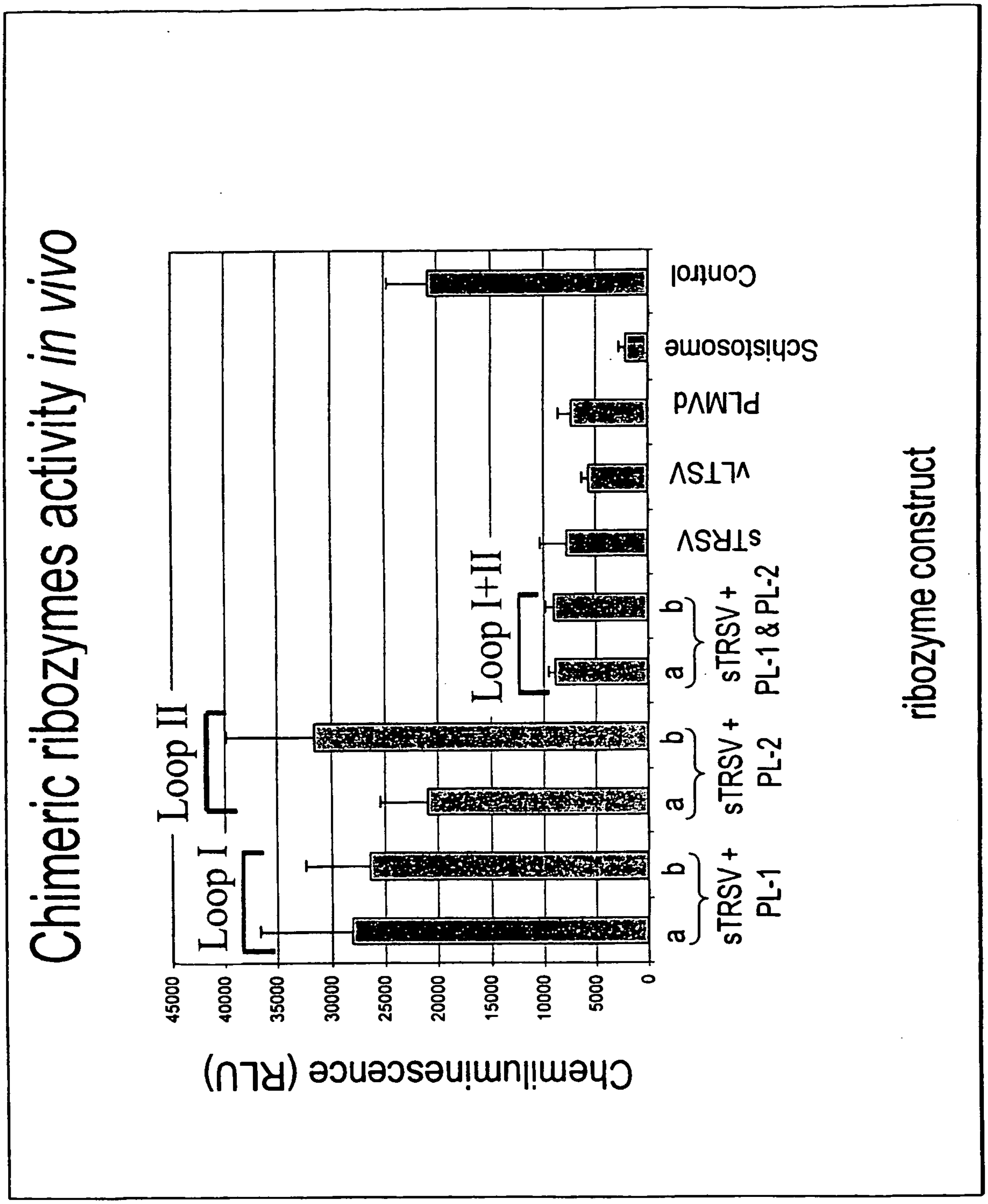

Chimeric ribozymes activity in vivo
Chemiluminescence (RLU)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Loop I
Loop II
Loop I+II
a
b
sTRSV +
PL-1
a
b
sTRSV +
PL-2
a
b
sTRSV +
PL-1 & PL-2
sTRSV
vLTSV
PLMVd
Schistosome
Control
ribozyme construct

FIGURE 10

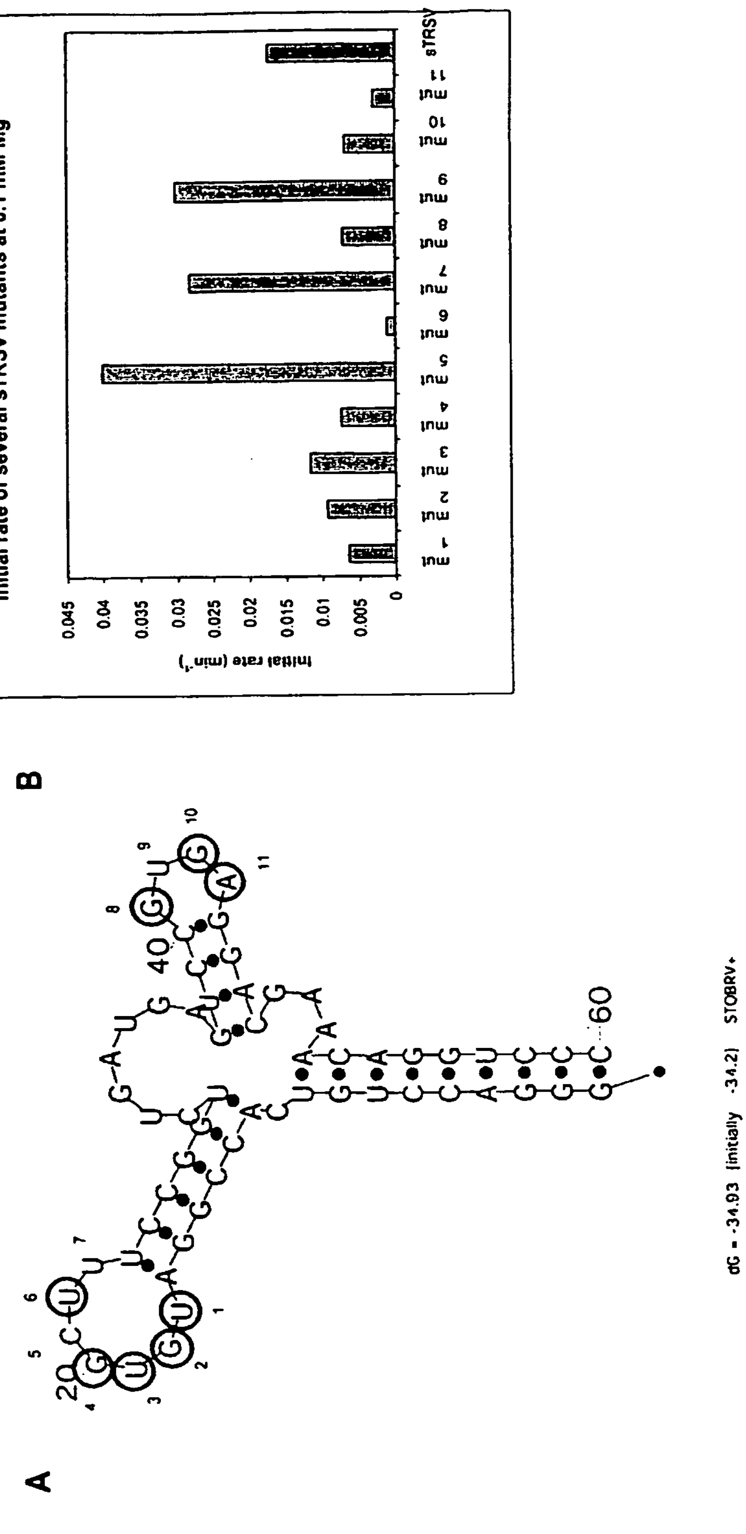

A
B
Initial rate of several sTRSV mutants at 0.1 mM Mg2+
initial rate (min-1)
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
mut 1
mut 2
mut 3
mut 4
mut 5
mut 6
mut 7
mut 8
mut 9
mut 10
mut 11
sTRSV
dG = -34.93 [initially -34.2] STOBRV+

Figure 11

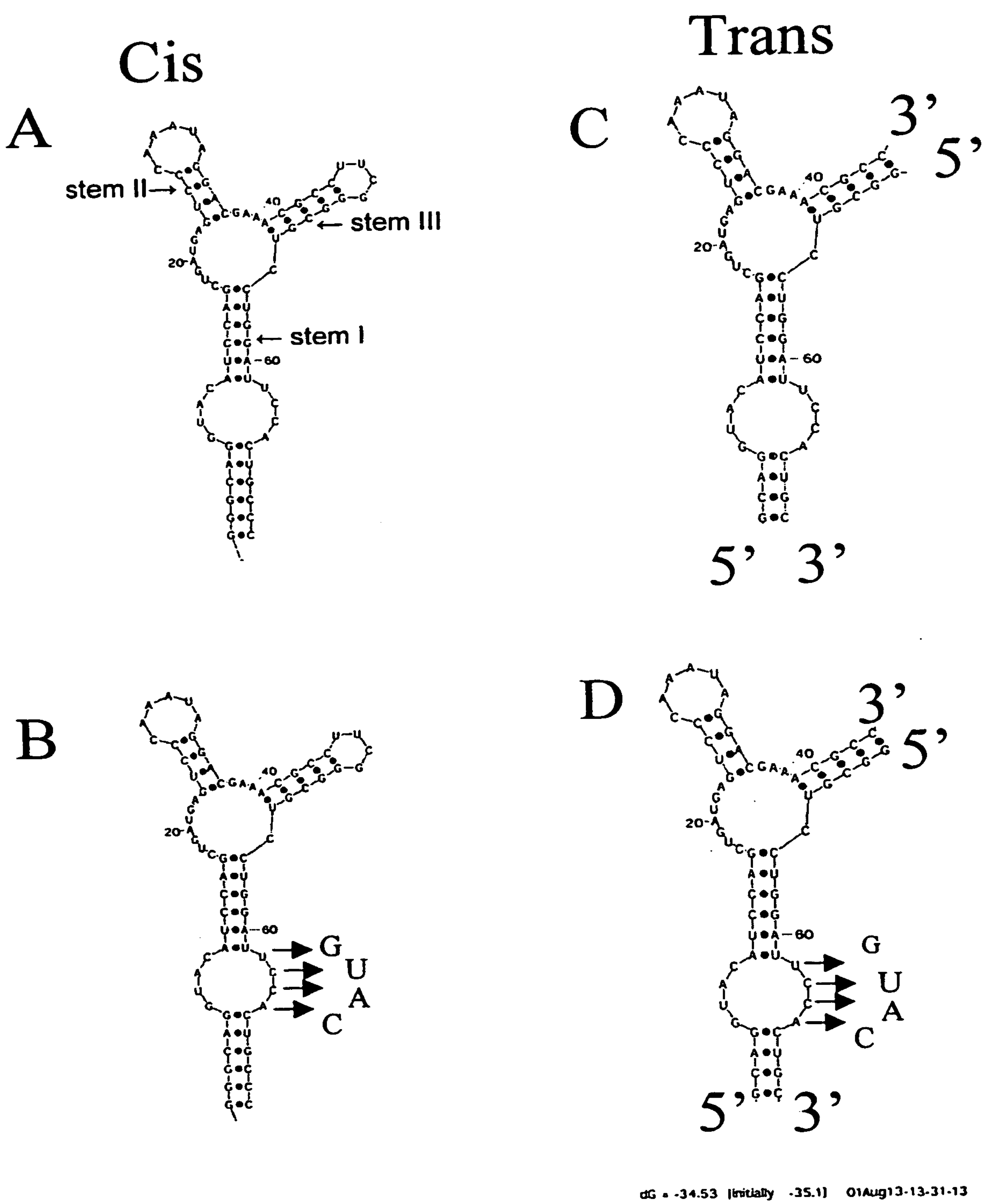
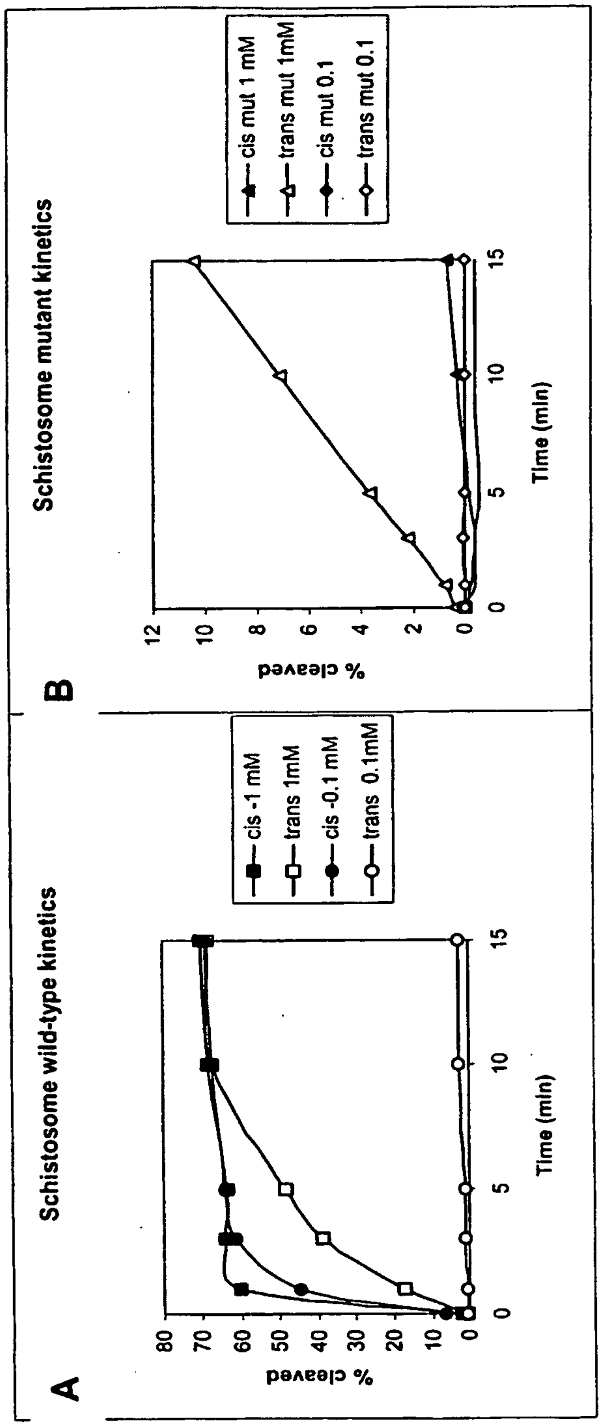

Cis
Trans
A
stem II
stem III
stem I
B
G
U
A
C
C
3'
5'
5'
3'
D
3'
5'
G
U
A
C
5'
3'
dG = -34.53 [initially -35.1] 01Aug13-13-31-13

FIGURE 12


A
Schistosome wild-type kinetics
% cleaved
Time (min)
cis -1 mM
trans 1mM
cis -0.1 mM
trans 0.1mM
B
Schistosome mutant kinetics
% cleaved
Time (min)
cis mut 1 mM
trans mut 1mM
cis mut 0.1
trans mut 0.1

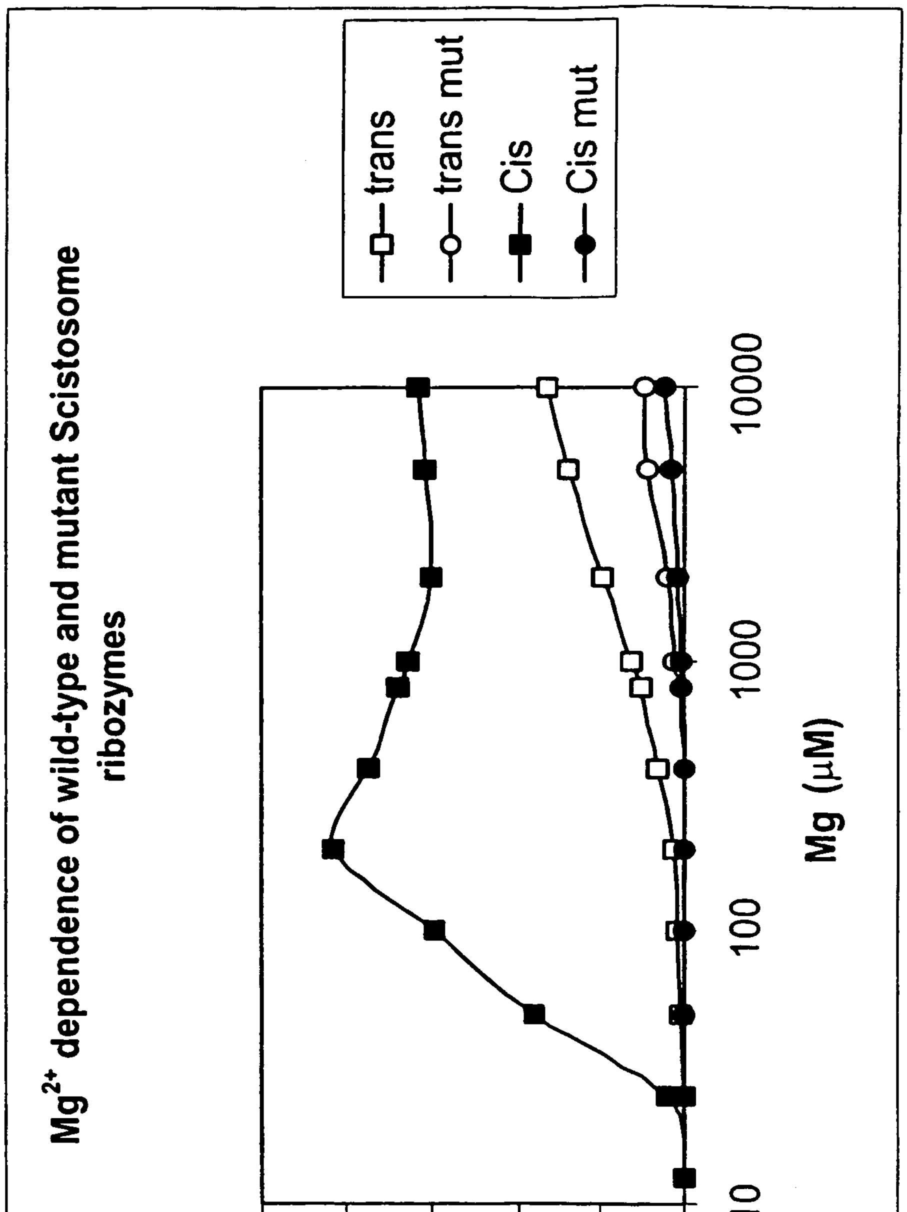

Mg^{2+} dependence of wild-type and mutant Scistosome ribozymes
% cleaved in 1 min
50.0
40.0
30.0
20.0
10.0
0.0
10
100
1000
10000
Mg (μM)
trans
trans mut
Cis
Cis mut

FIGURE 13

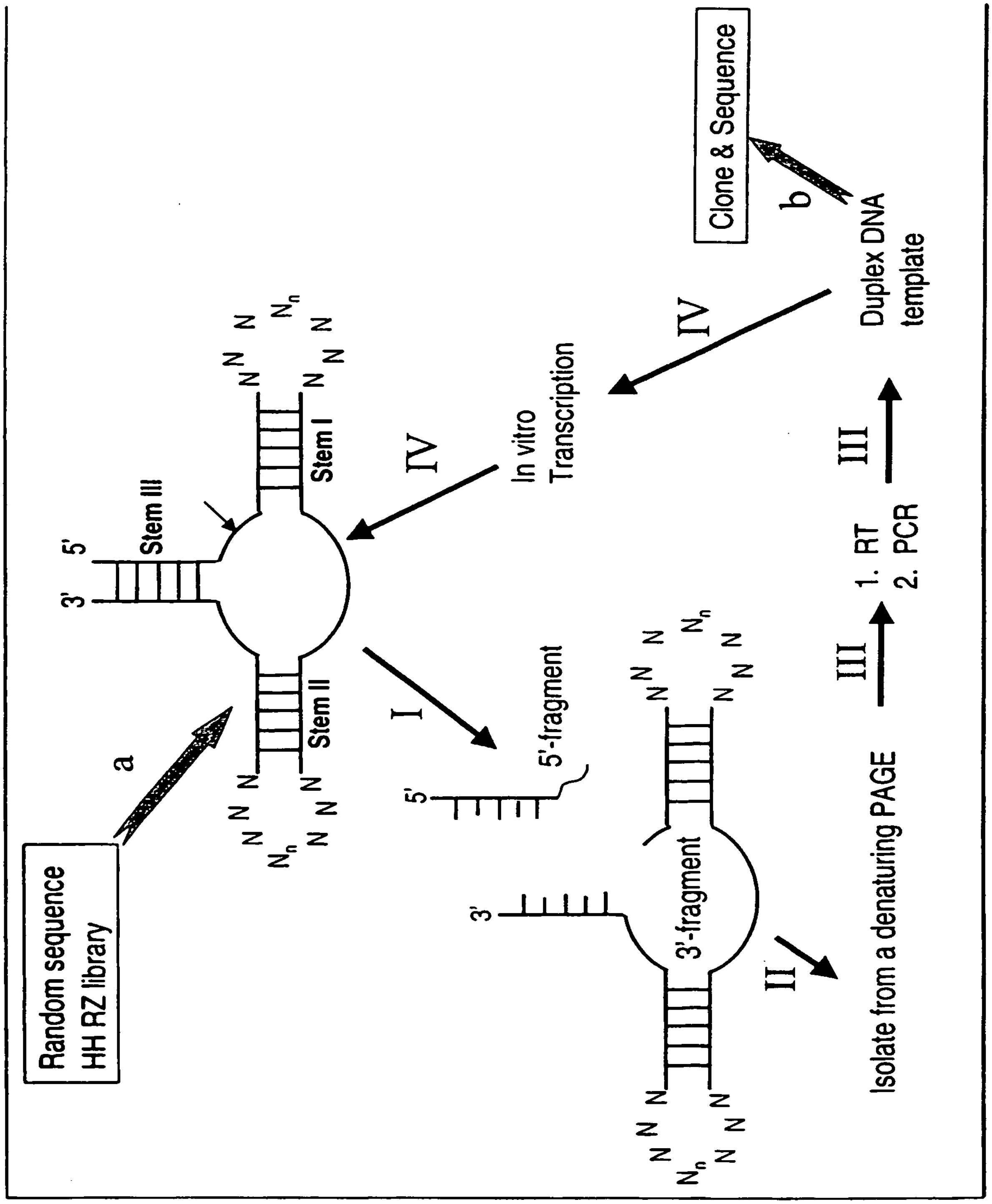

Random sequence HH RZ library
a
3' 5'
Stem III
Stem I
Stem II
I
5'-fragment
5'
3'
3'-fragment
II
Isolate from a denaturing PAGE
III
1. RT
2. PCR
III
Duplex DNA template
b
Clone & Sequence
IV
In vitro Transcription
IV

FIGURE 14

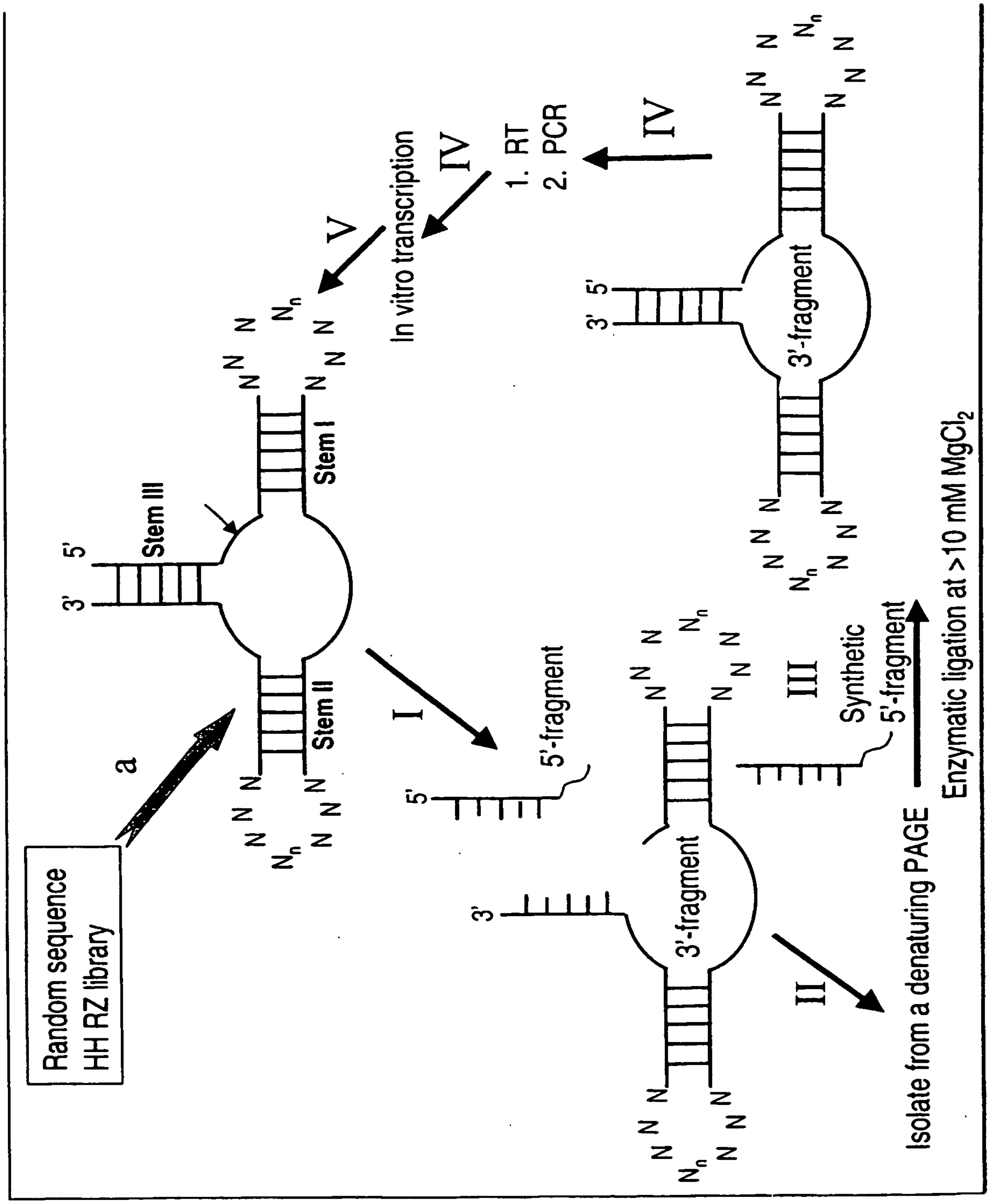

Random sequence HH RZ library
a
3'
5'
Stem III
Stem II
Stem I
I
5'-fragment
3'-fragment
II
Isolate from a denaturing PAGE
III
Synthetic 5'-fragment
Enzymatic ligation at >10 mM $MgCl_2$
IV
1. RT
2. PCR
In vitro transcription
V

FIGURE 15

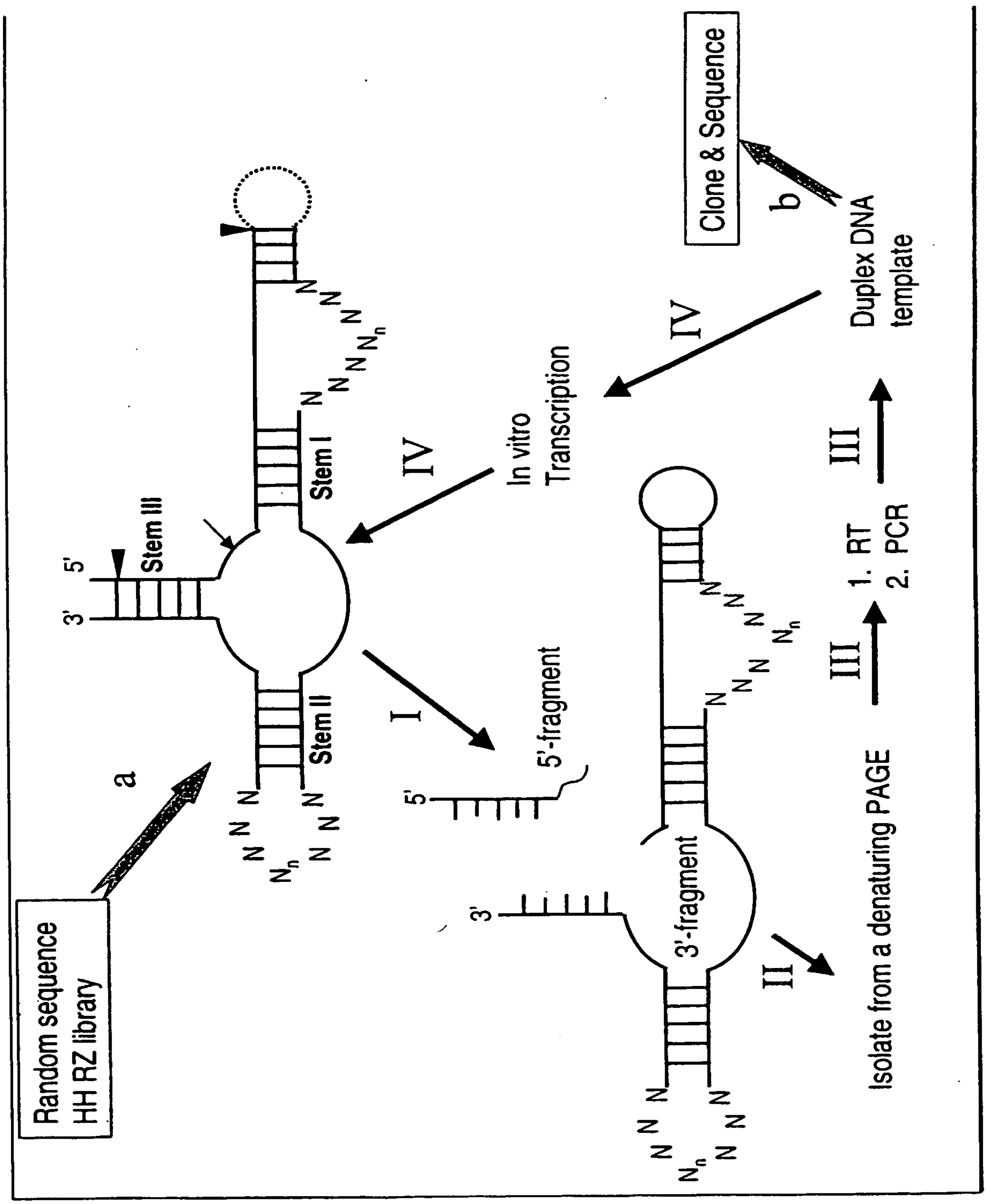

Random sequence HH RZ library
a
3'
5'
Stem III
Stem II
Stem I
N
N_n
IV
In vitro Transcription
I
5'-fragment
5'
3'
3'-fragment
II
Isolate from a denaturing PAGE
III
1. RT
2. PCR
Duplex DNA template
b
Clone & Sequence

FIGURE 16

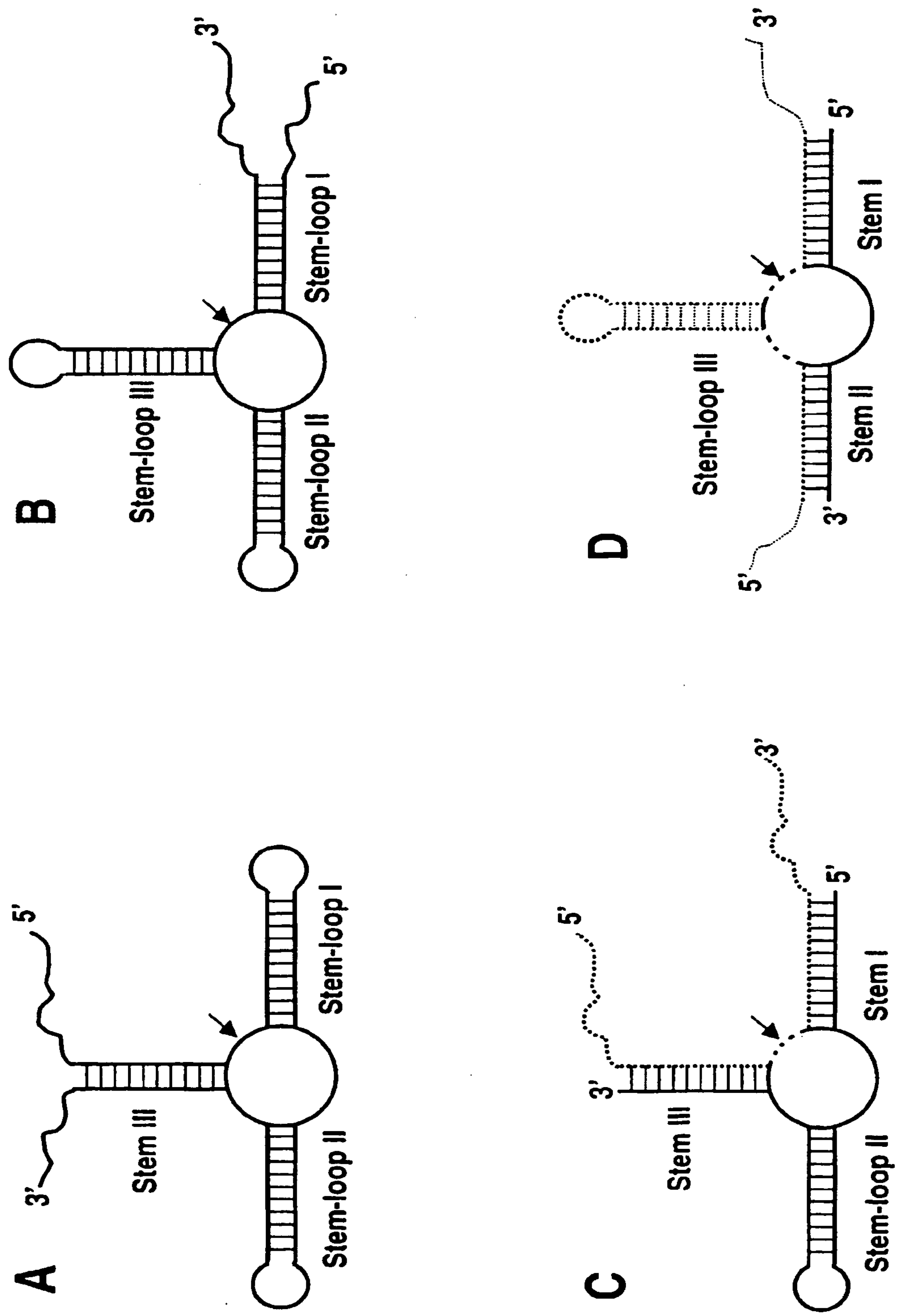

A
5'
3'
Stem III
Stem-loop I
Stem-loop II
B
3'
5'
Stem-loop III
Stem-loop I
Stem-loop II
C
5'
3'
3'
5'
Stem III
Stem I
Stem-loop II
D
3'
5'
5'
3'
Stem-loop III
Stem I
Stem II

Figure 17

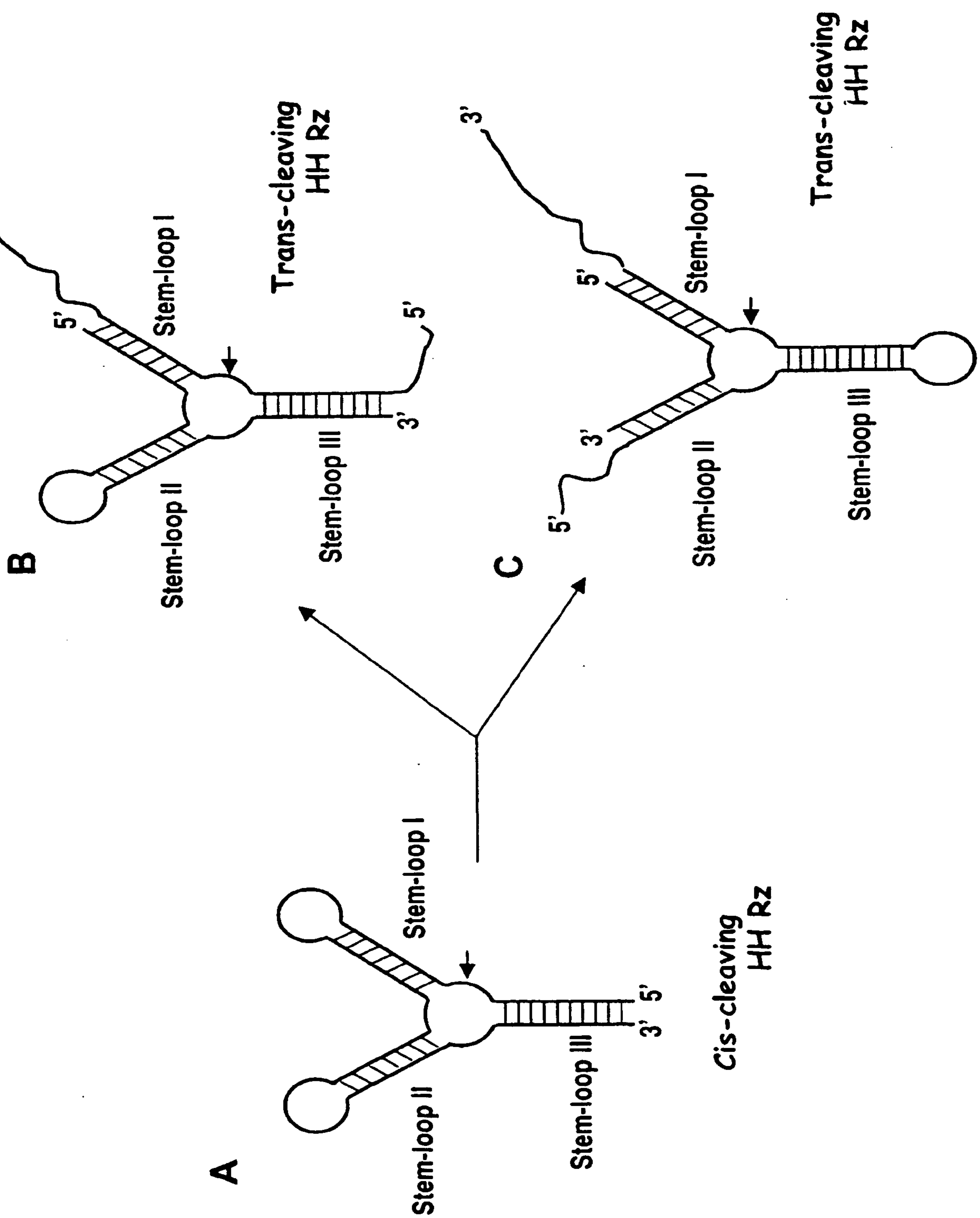

A
Stem-loop I
Stem-loop II
Stem-loop III
3'
5'
Cis-cleaving
HH Rz
B
Stem-loop I
Stem-loop II
Stem-loop III
5'
3'
3'
5'
Trans-cleaving
HH Rz
C
Stem-loop I
Stem-loop II
Stem-loop III
5'
3'
3'
5'
Trans-cleaving
HH Rz

Figure 18

10/517636

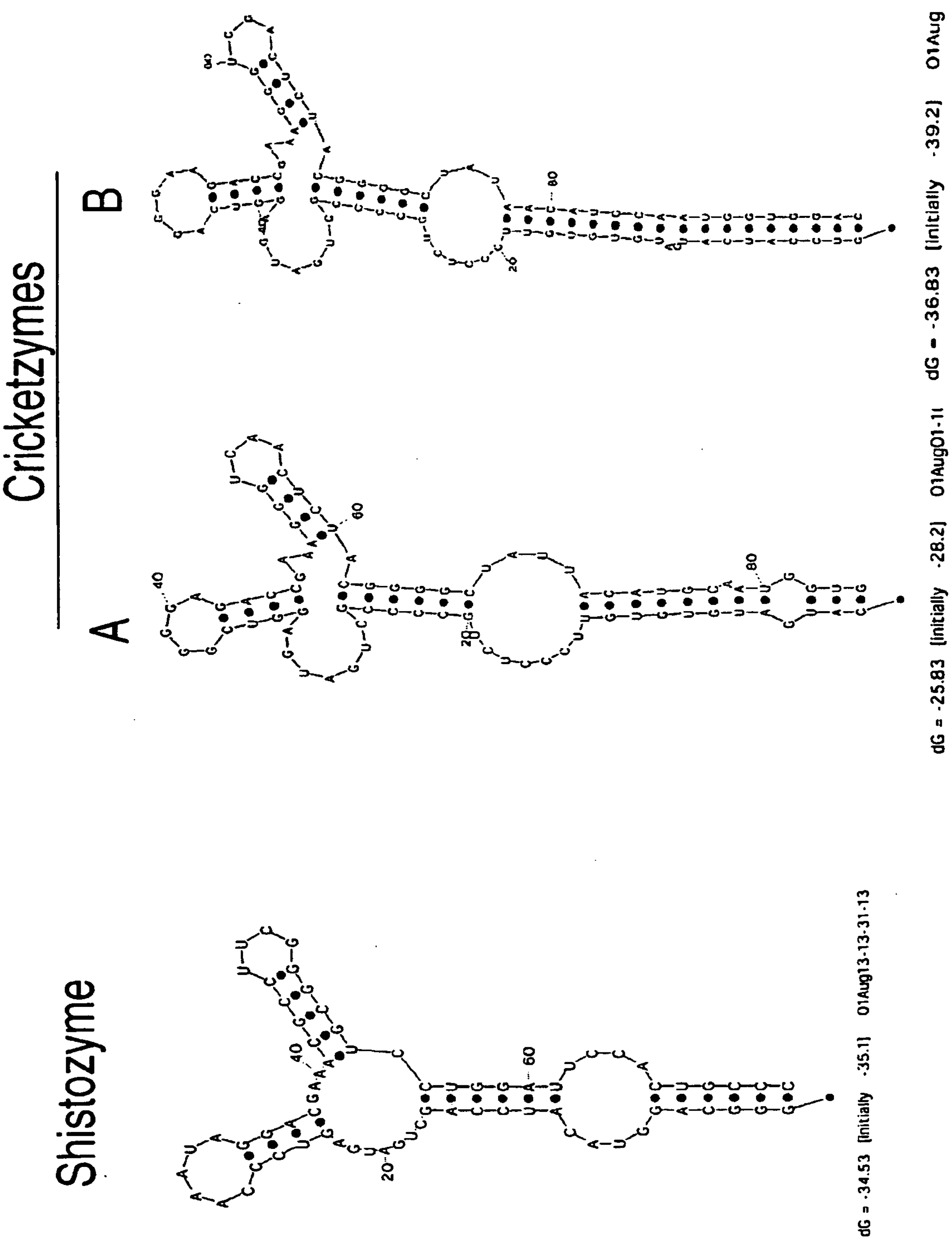


Figure 19

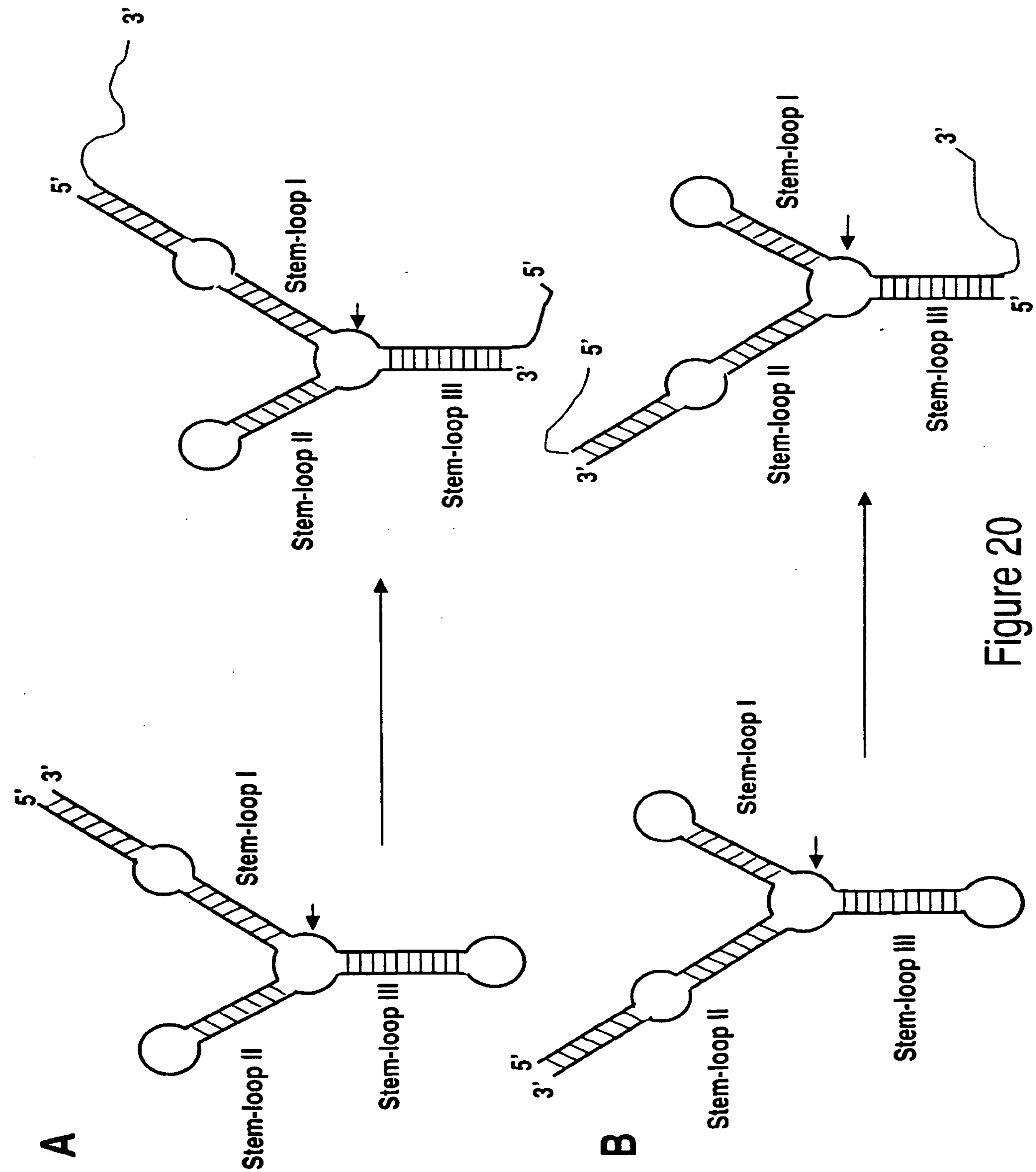


Figure 20

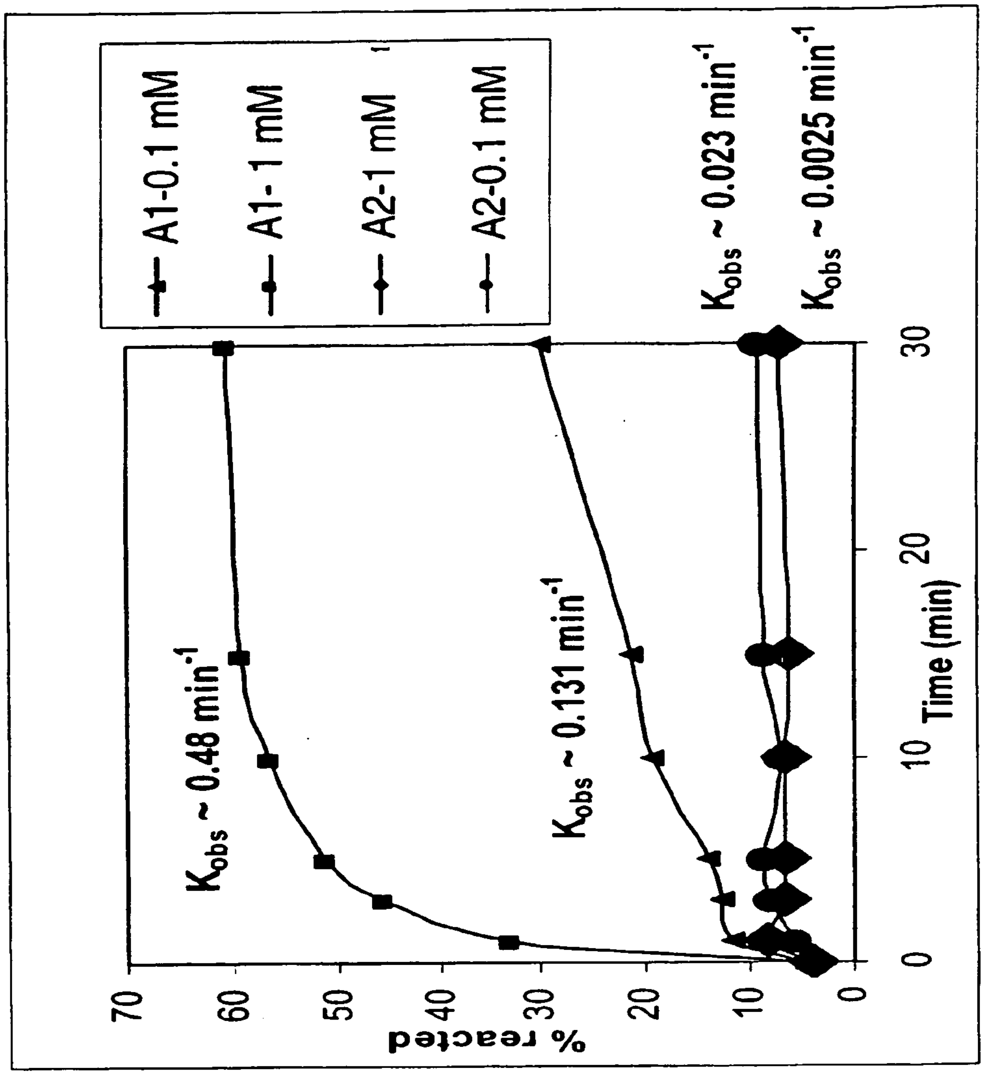
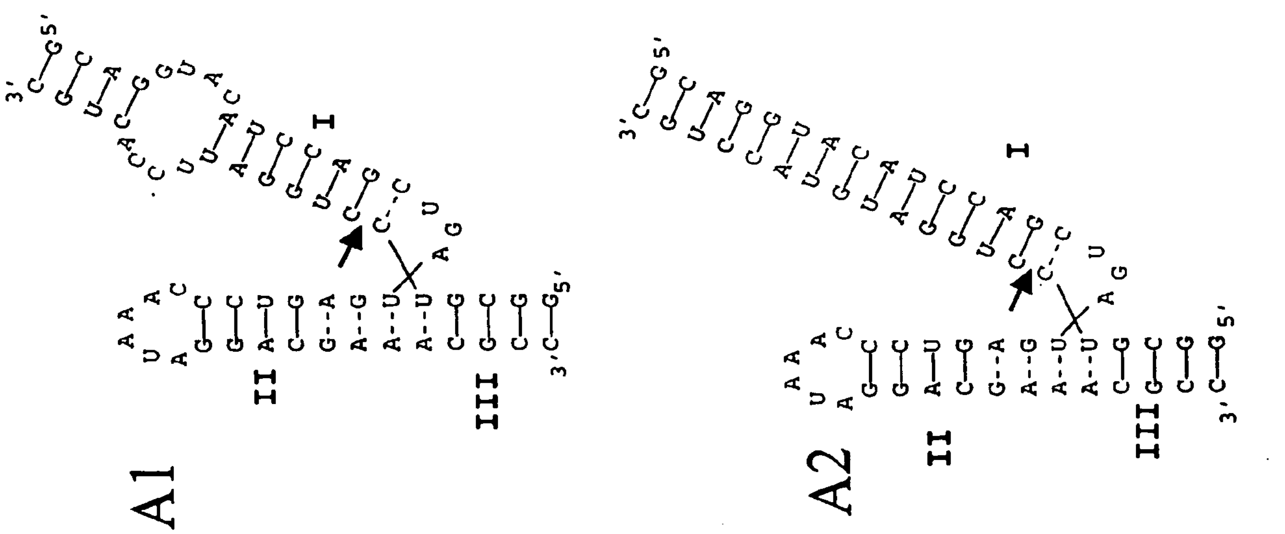
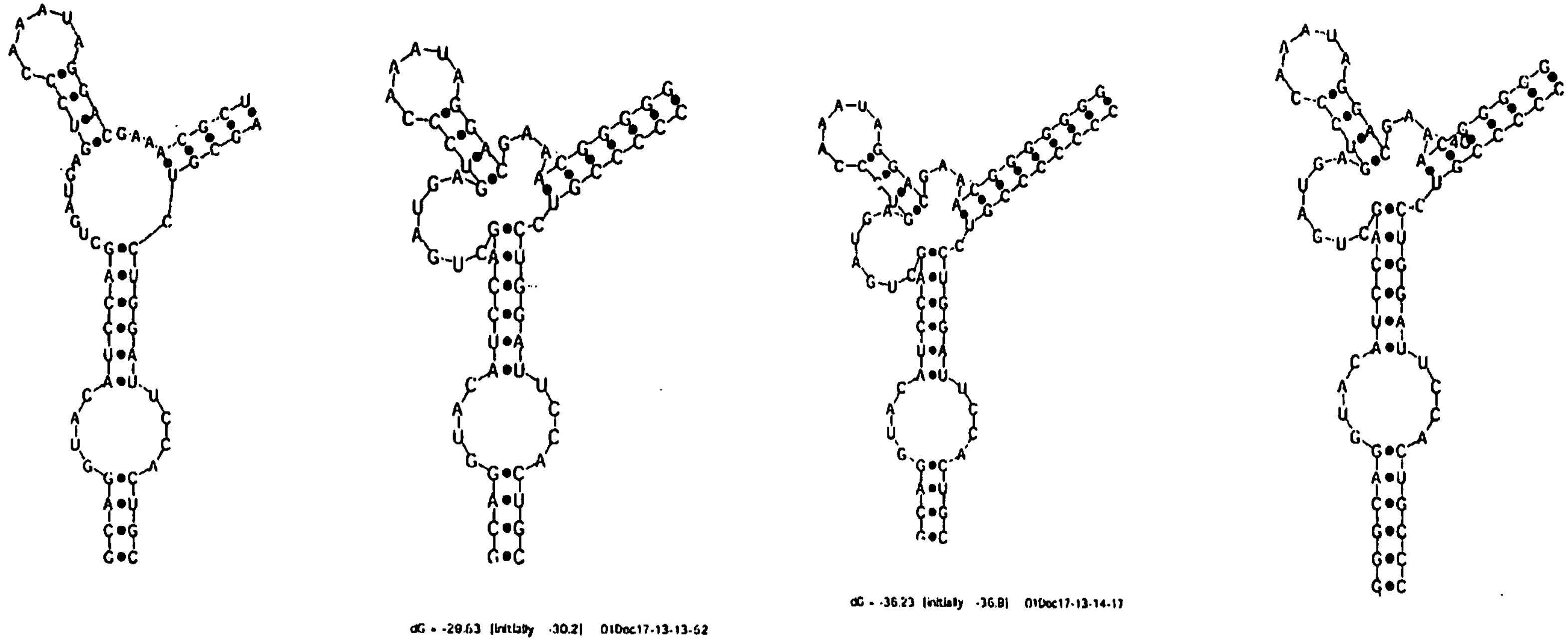

A1-0.1 mM
A1- 1 mM
A2-1 mM
A2-0.1 mM
K$_{obs}$ ~ 0.48 min^{-1}
K$_{obs}$ ~ 0.131 min^{-1}
K$_{obs}$ ~ 0.023 min^{-1}
K$_{obs}$ ~ 0.0025 min^{-1}
% reacted
Time (min)
0
10
20
30
40
50
60
70

Figure 21


A1
A2
I
II
III
3'
5'

10/517638



ΔG -21.9	-29.6	-36.2	-36.8
TCHR-5:4	TCHR-7:4	TCHR-9:4	TCHR-7:6

Figure 22

Kinetics of different TCHRs in 0.1 mM $MgCl_2$

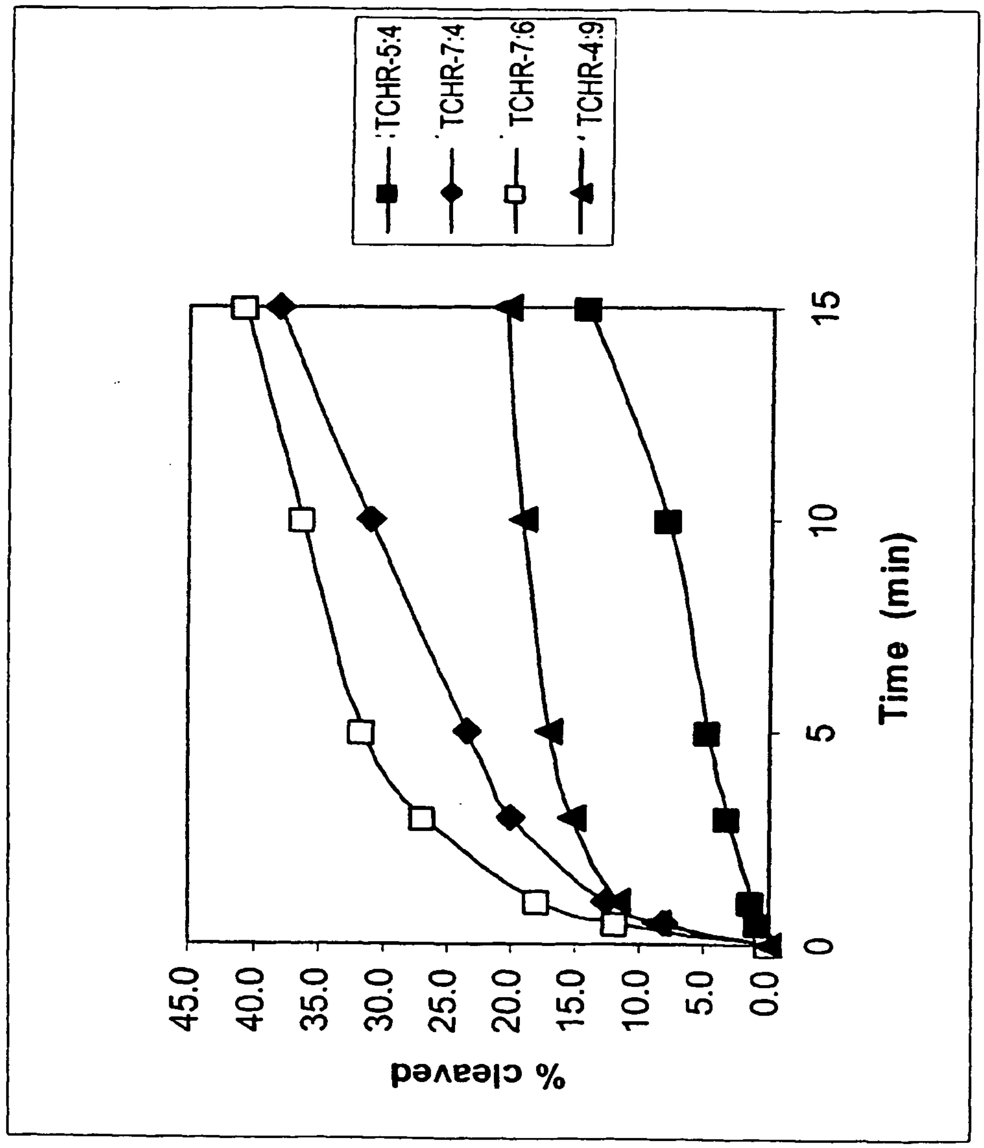
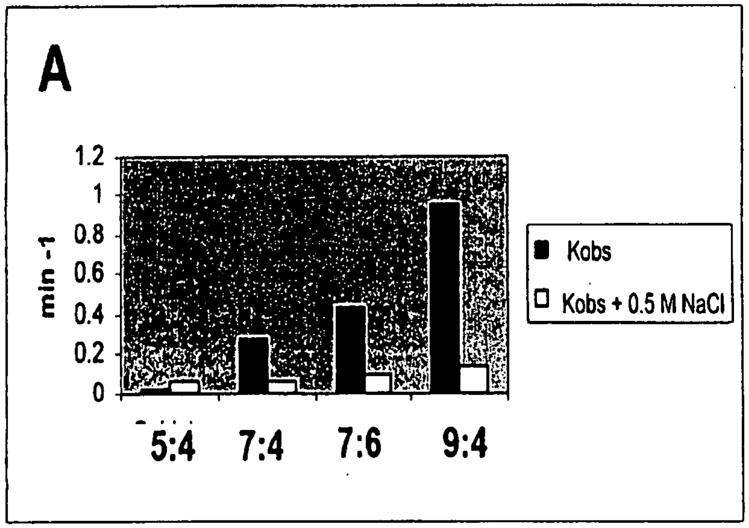


Figure 23



B

	Kobs	Relative Activity
TCHR-5:4	0.02	1
TCHR-7:4	0.29	15
TCHR-7:6	0.43	22
TCHR-9:4	0.95	49

Figure 24

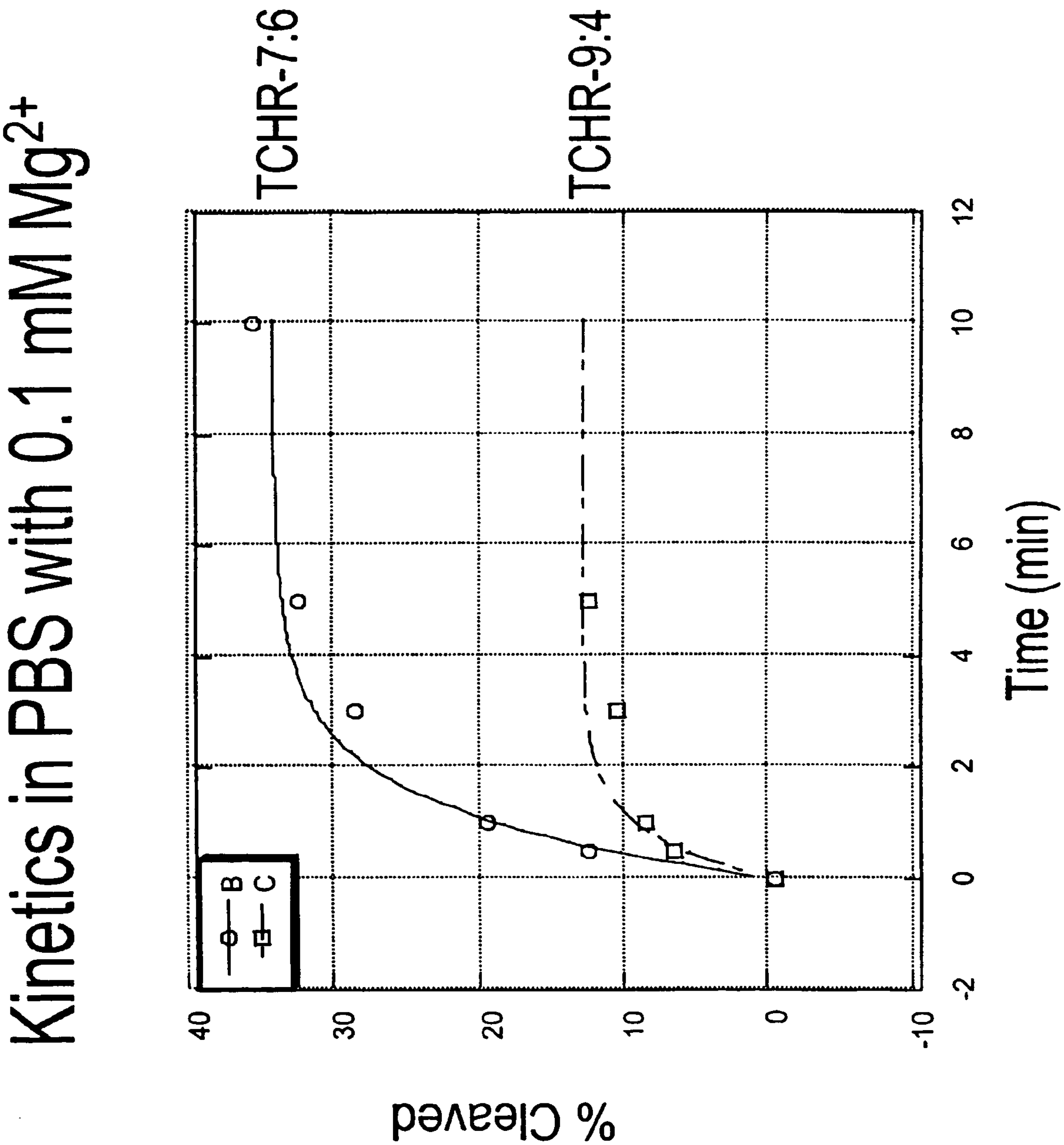
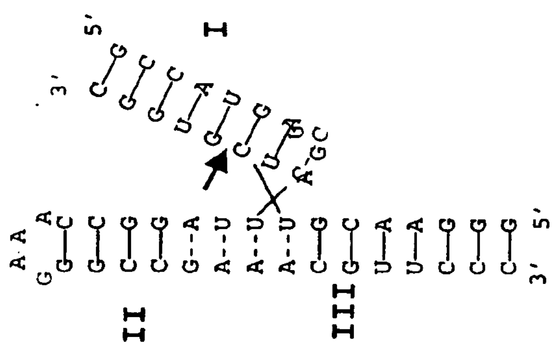

Kinetics in PBS with 0.1 mM Mg2+
TCHR-7:6
TCHR-9:4
B
C
% Cleaved
40
30
20
10
0
-10
-2
0
2
4
6
8
10
12
Time (min)

Figure 25



HH16

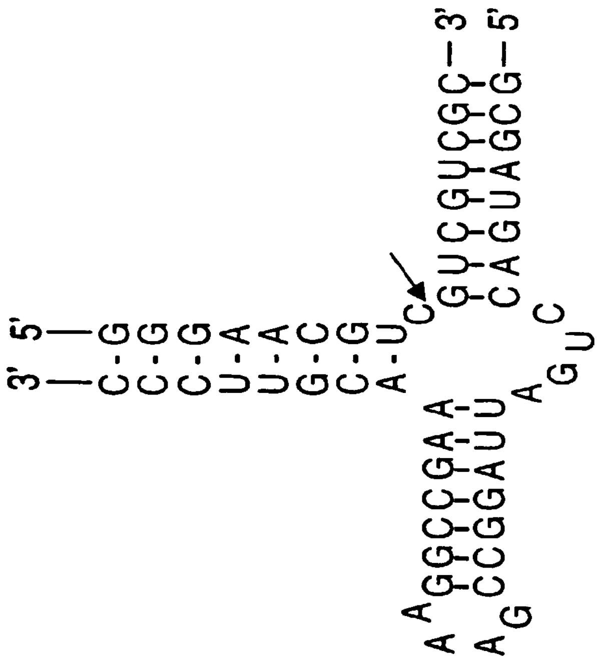


Figure 26

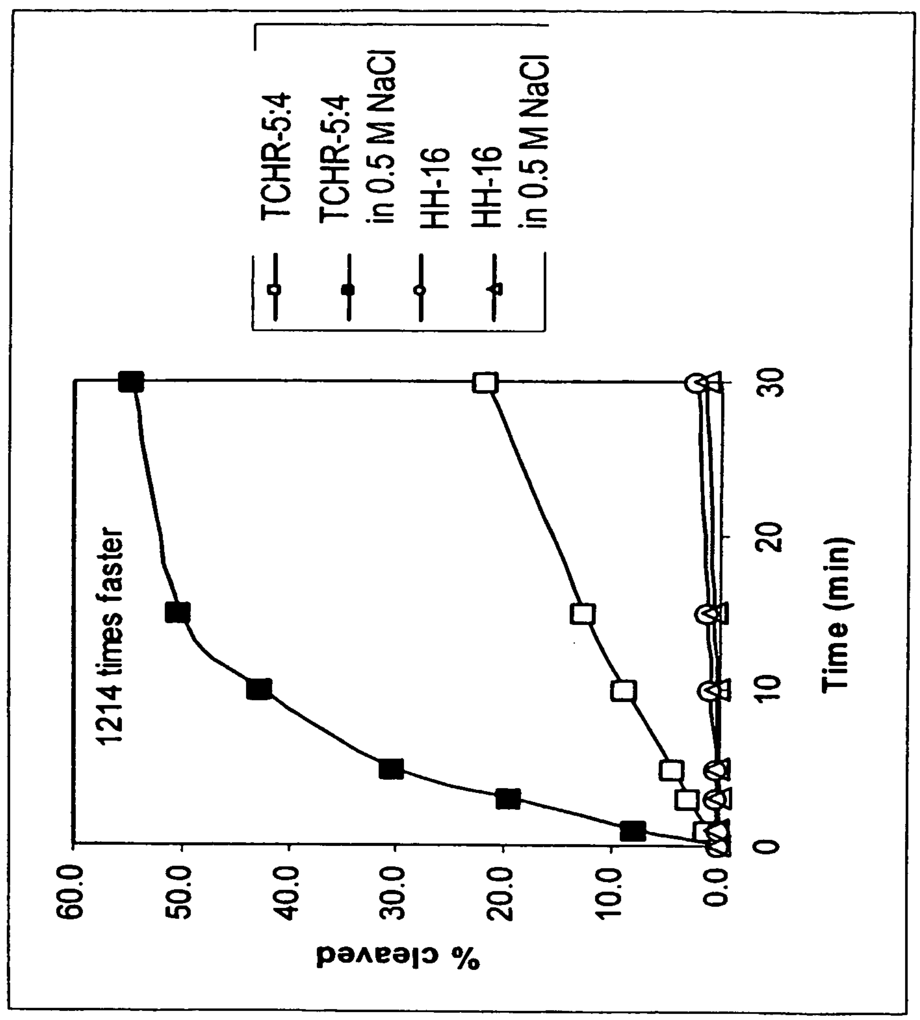

1214 times faster
% cleaved
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
10
20
30
Time (min)
TCHR-5:4
TCHR-5:4 in 0.5 M NaCl
HH-16
HH-16 in 0.5 M NaCl

Figure 27

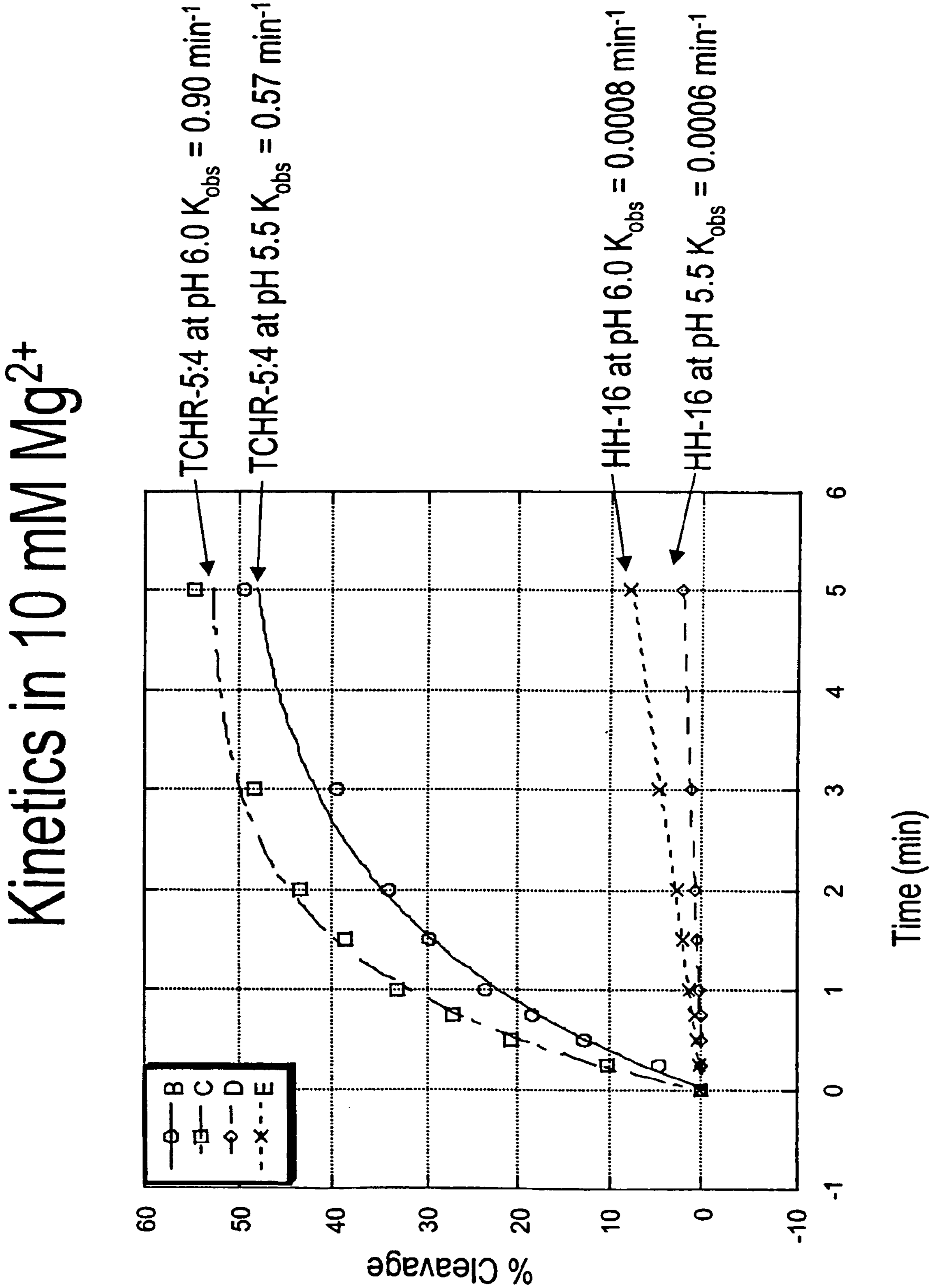

Kinetics in 10 mM Mg2+
TCHR-5:4 at pH 6.0 Kobs = 0.90 min-1
TCHR-5:4 at pH 5.5 Kobs = 0.57 min-1
HH-16 at pH 6.0 Kobs = 0.0008 min-1
HH-16 at pH 5.5 Kobs = 0.0006 min-1
B
C
D
E
% Cleavage
Time (min)

Figure 28

Cleavage of TCHR-5:4 and HH16 in the presence of LiCl

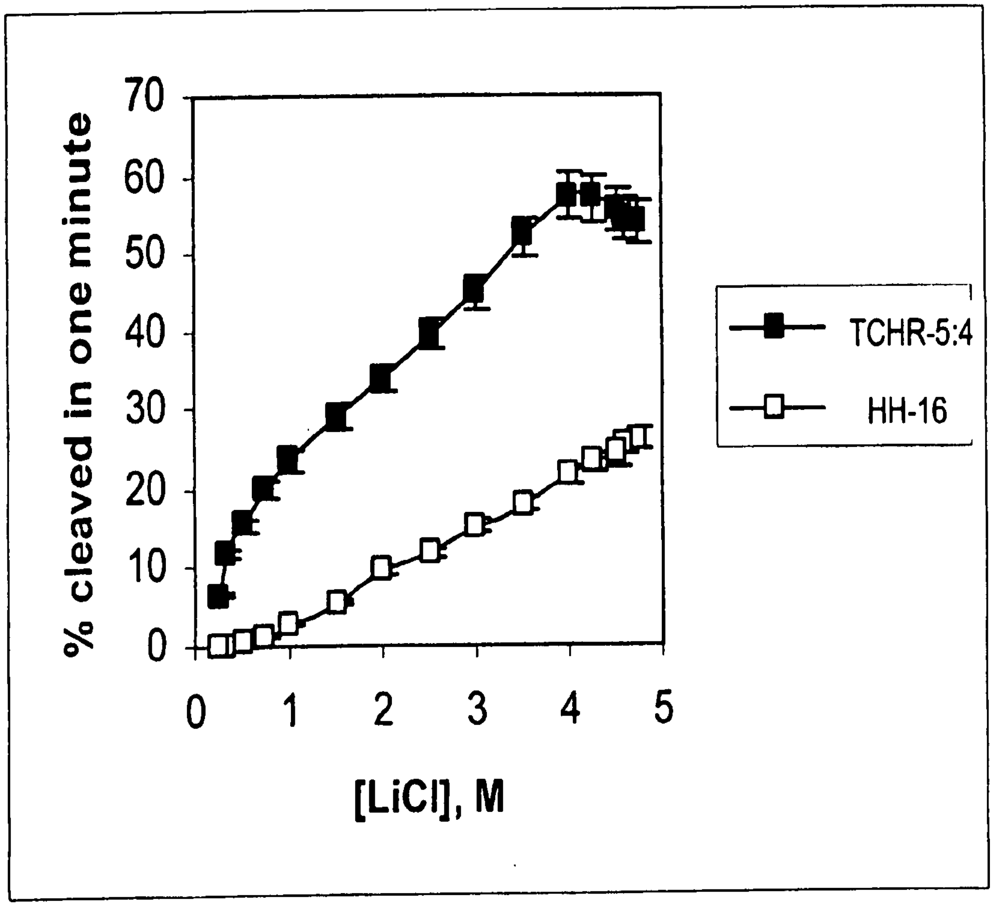


Figure 29

pH dependence of TCHR-5:4 and HH16 at 0.1 mM Mg^{2+}

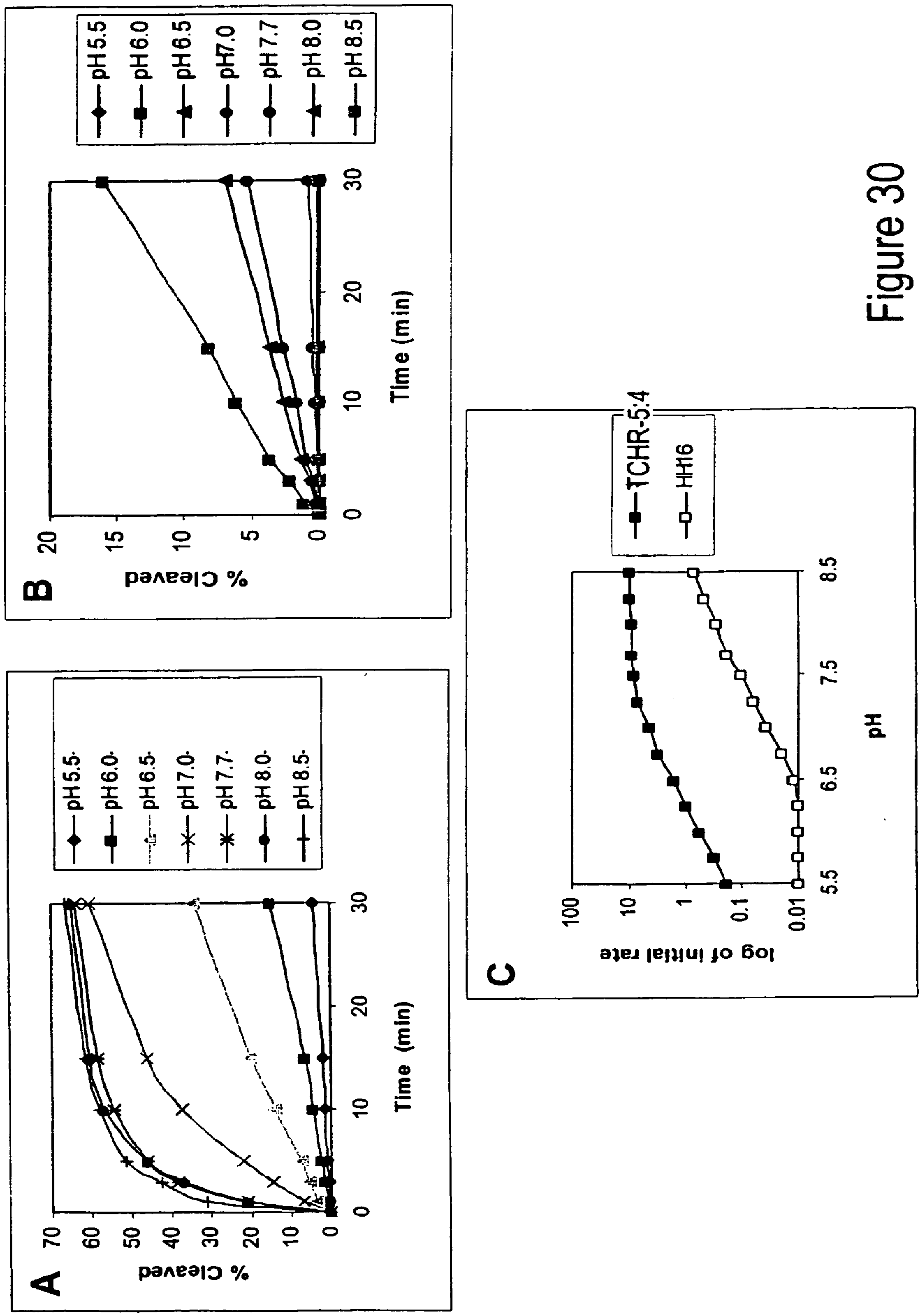


Figure 30

Temperature dependence at 0.1 mM Mg^{2+}

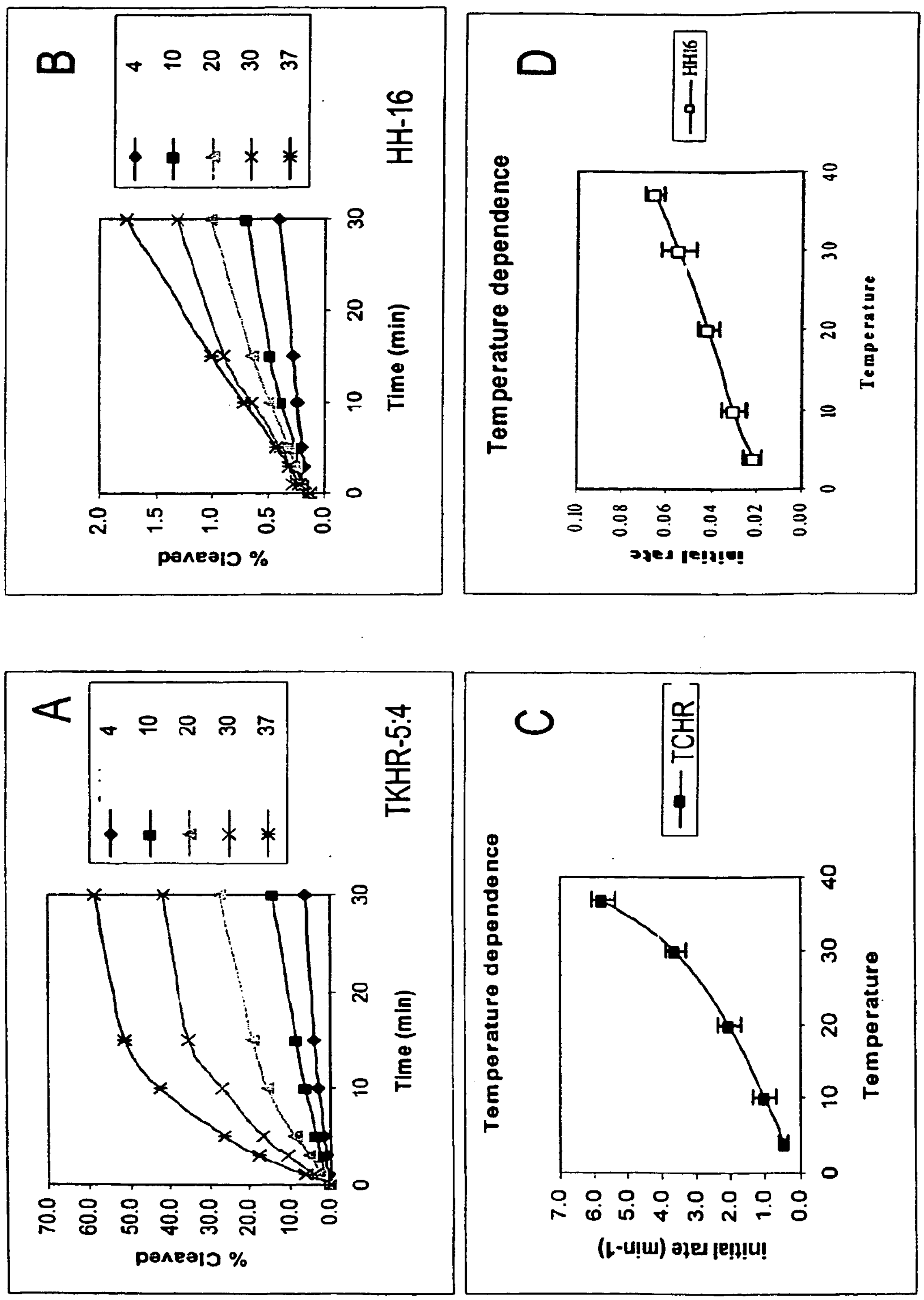


Figure 31

Mg^{2+} dependence of TCHR-5:4 and HH16 at different pH

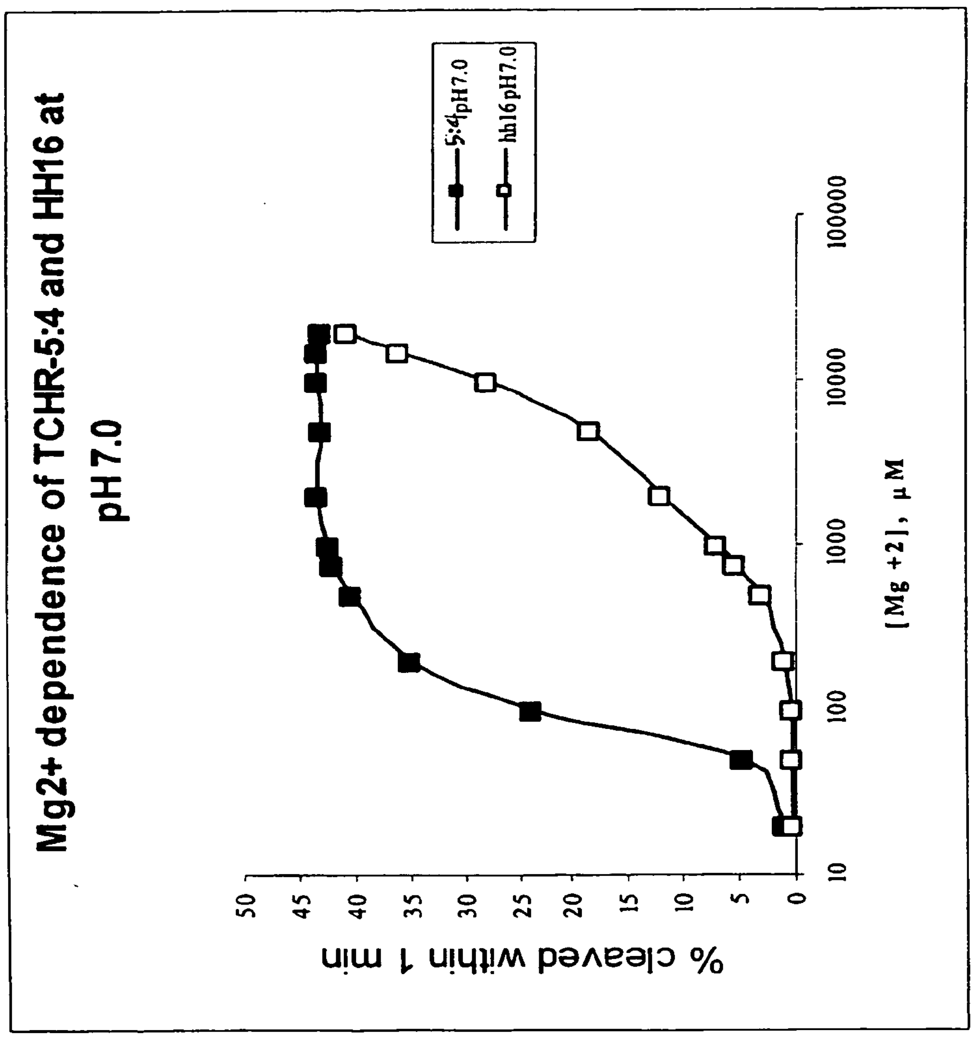


Figure 32

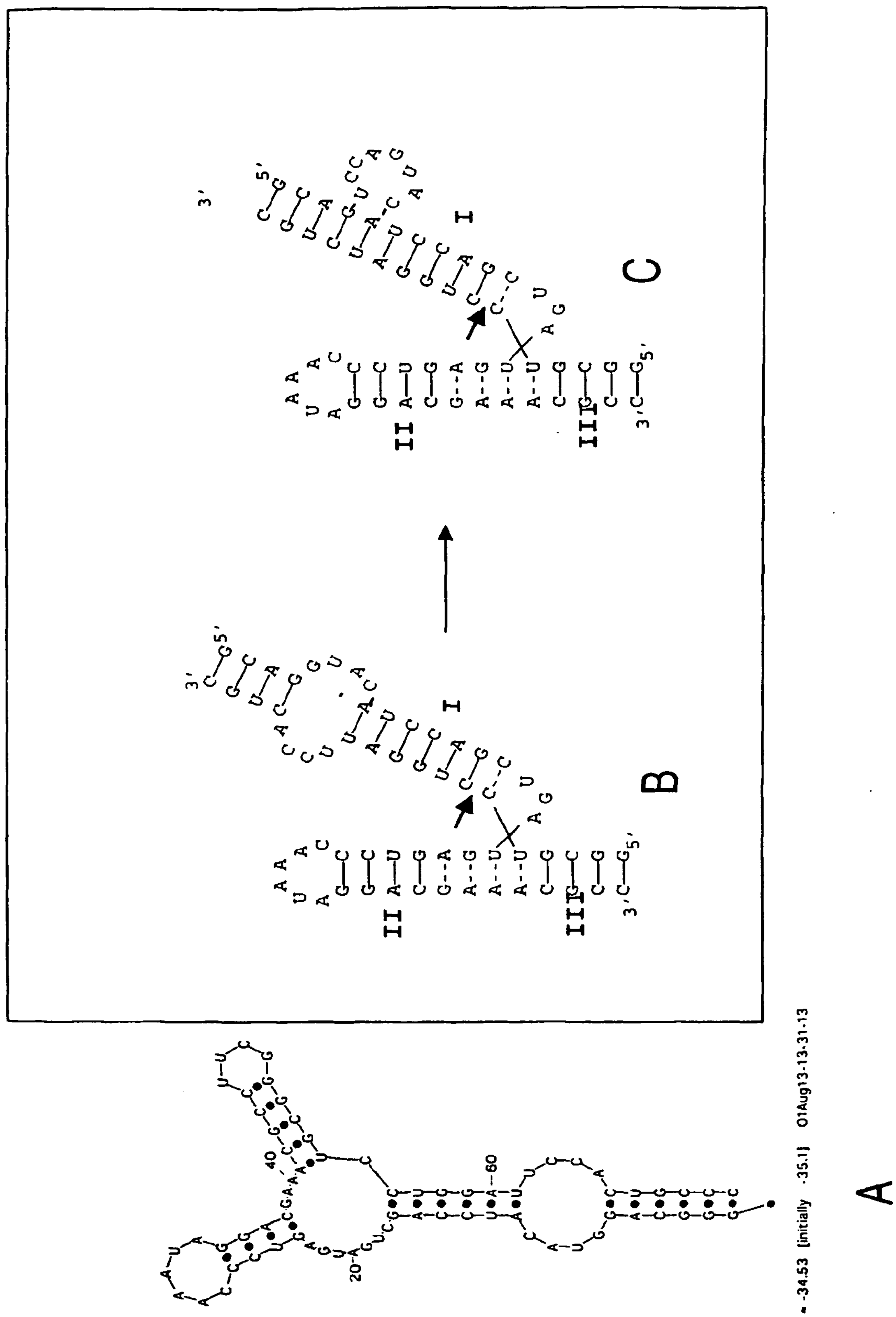


Figure 33

Changing the position of the internal bulge resulted in loss of activity

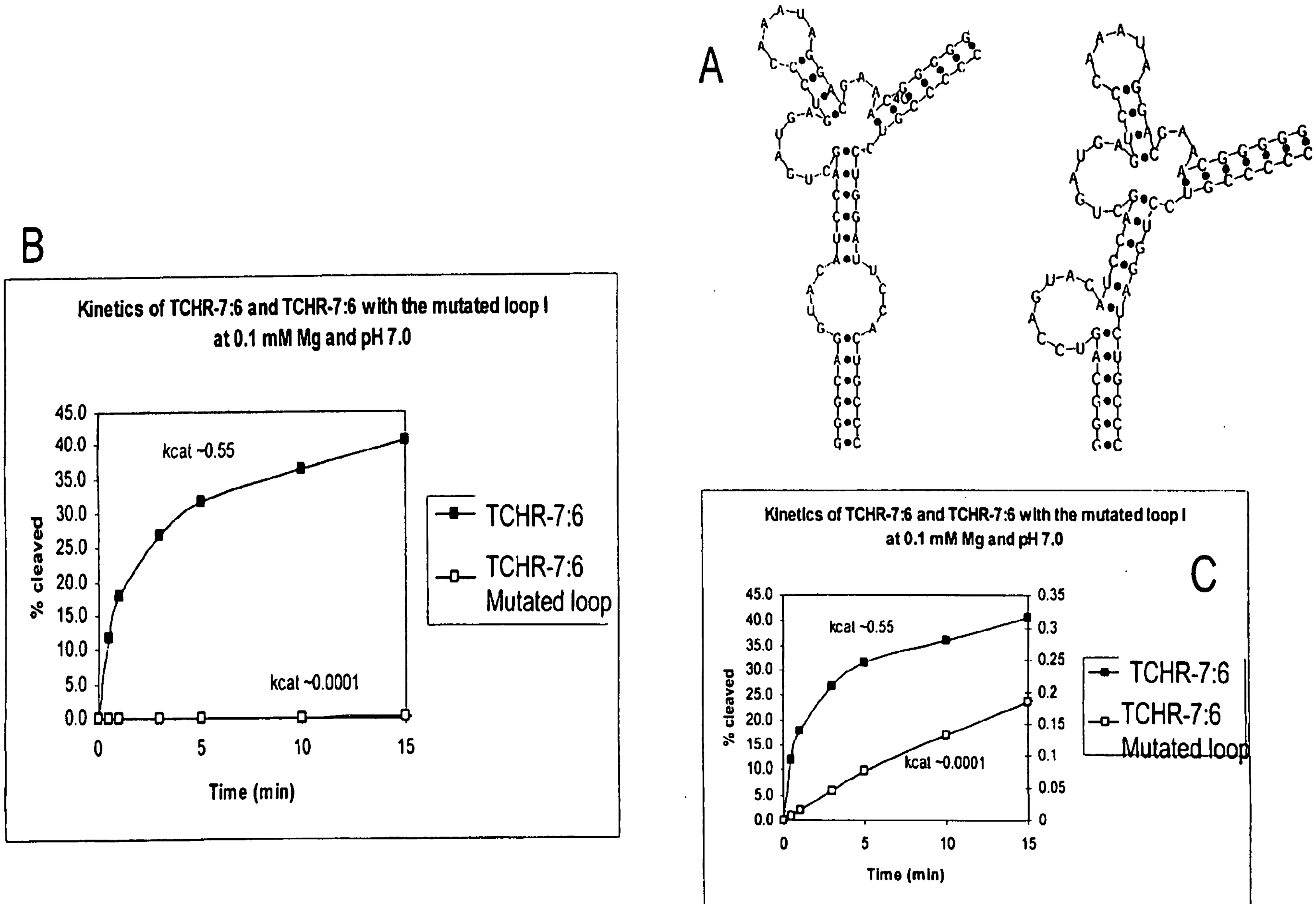
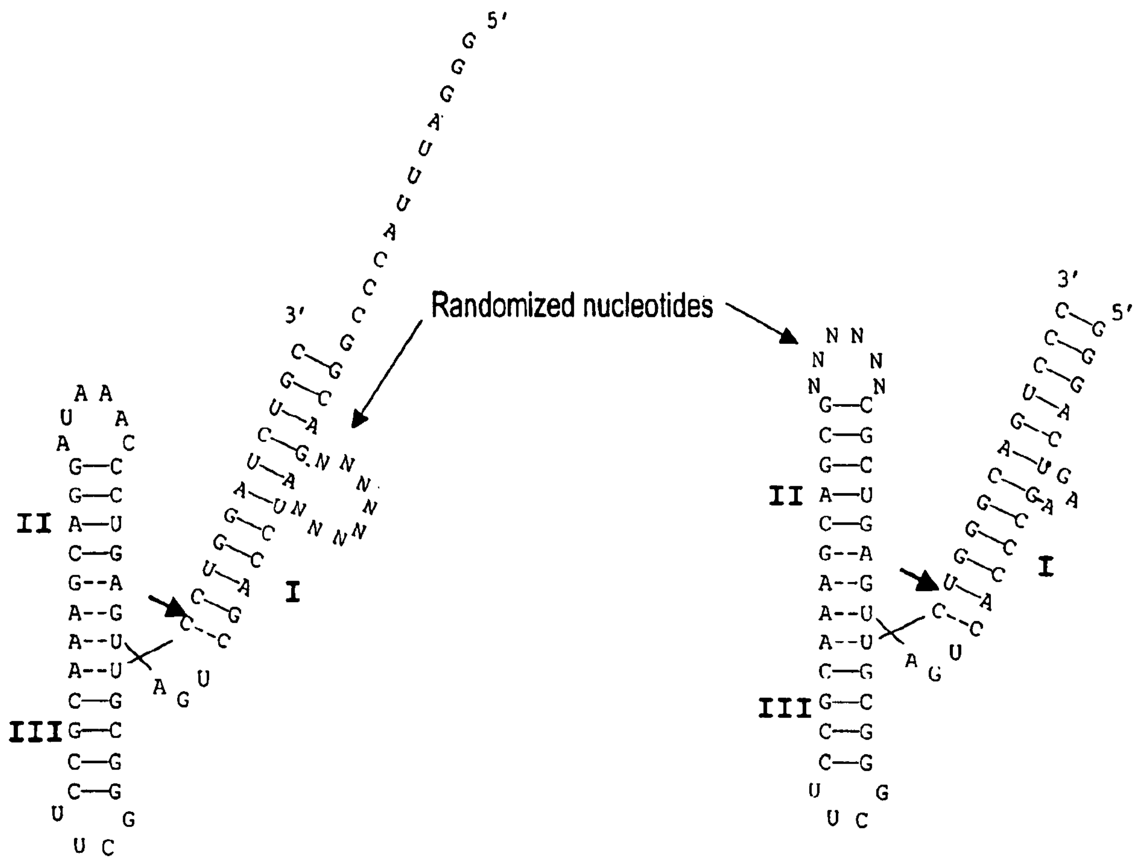


Figure 34

Design of randomized libraries for isolating trans-cleaving kissing HH Rzs



Stem I randomized
(Library A)

Stem II randomized
(Library B)

Figure 35

Figure 36

Template A

5'-GGGATTTACCCGGCAGNNNNNNNNATCCAGCTGATGAGTCCCAAATAGGACGAAACGCCTTCGGGCGTCCTGGATCTGC-3' (SEQ ID NO: __)

T7-A

5'-TAATACGACTCACTATAGGGATTTACCCGGCAG-3' (SEQ ID NO: __)

RT-A

5'-GCAGATCCAGGACGCCCG-3' (SEQ ID NO: __)

Antisense-A

5'-GTCCTATTTGGGACTCATCAGCTGGAT-3' (SEQ ID NO: __)

Template B

5'-GGGACTTAAGCCCACTGATGAGTCGCNNNNNNGCGACGAAACGCCTTCGGGCGTCTGGGCAGTCCC-3' (SEQ ID NO: __)

T7-B

5'-TAATACGACTCACTATAGGGACTTAAGCCCACTG-3' (SEQ ID NO: __)

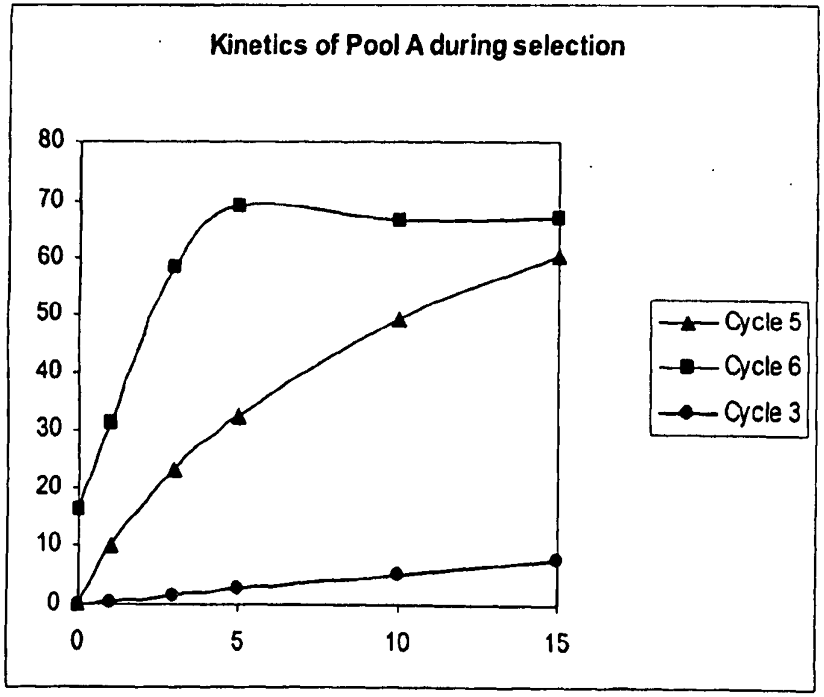
RT-B

5'-GGGACTGCCCAGACGCCCGAAGGCGTTTC-3' (SEQ ID NO: __)

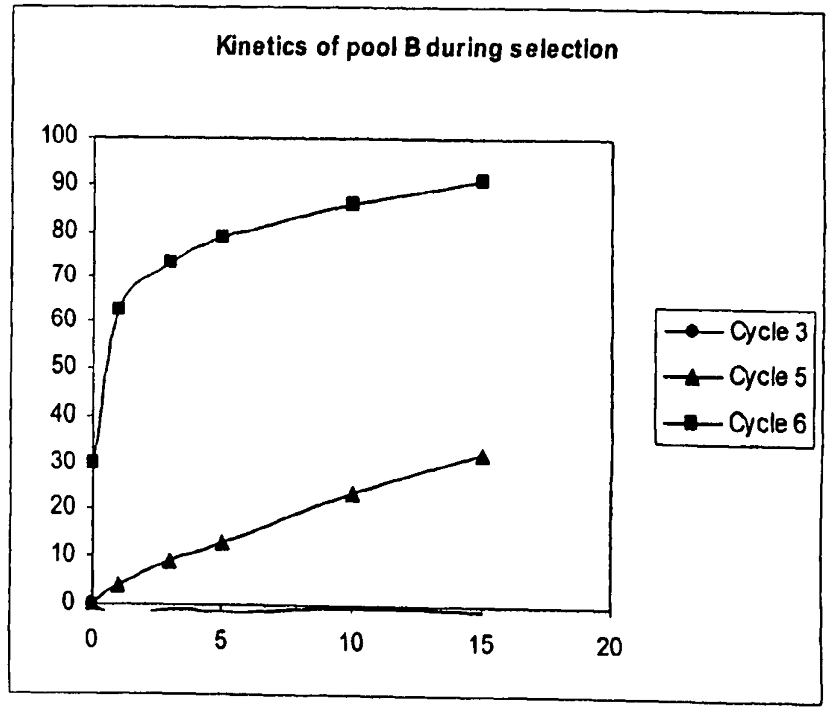
Antisense-B

5'-GCGACTCATCAGTGGGCTTAAGTCCC-3' (SEQ ID NO: __)

Progress of in vitro selection of trans-cleaving hammerhead ribozymes



Library A



Library B

Figure37

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A

Sequence #	Sequence(5' - 3')				
	GGGAUUUACCCGGCAG	NNNNNNNN	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCCUUCGGGCGUCCUGGAUCUGC		(SEQ ID NO:__)
2002062995	GGGAUUUACCCGGCAG	GGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGC	UUCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002062997	GAUUUACCCGGCAG	GGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063002	GGGAUUUACCCGGCAG	GGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063004	GGGAUUUACCCGGCAG	GGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063022	GGGAUUUACCCGGCAG	GGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063036	GGGAUUUACCCGGCAG	GGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063041	GGGAUUUACCCGGCAG	GGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063053	GGGAUUUACCCGGCAG	GGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063068	GGGAUUUACCCGGCAG	GGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063045	GGGAUUUACCCGGCAG	GGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063047	GGGAUUUACCCGGCAG	GGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063021	UGAUUUACCCGGCAG	GGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063030	GGGAUUUACCCGGCAG	CGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063025	GGGAUUUACCCGGCAG	CGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063059	GGGAUUUACCCGGCAG	CGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002062994	GGGAUUUACCCGGCAG	CGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUG	(SEQ ID NO:__)
2002063006	GGGAUUUACCCGGCAG	CGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGG	(SEQ ID NO:__)
2002063048	GGGAUUUACCCGGCAG	UGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063003	GGGAUUUACCCGGCAG	UGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063017	GGGAUUUACCCGGCAG	UGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063058	GGGAUUUACCCGGCAG	UGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
200206306	GGGAUUUACCCGGCAG	AGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063070	GGGAUUUACCCGGCAG	AGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063065	GGGAUUUACCCGGCAG	AGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063024	GGGAUUUACCCGGCAG	AGGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGG	(SEQ ID NO:__)
2002063051	GGGAUUUACCCGGCAG	GCGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063044	GGGAUUUACCCGGCAG	GCGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063007	GGGAUUUACCCGGCAG	GAGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063009	GGGAUUUACCCGGCAG	GUGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063027	GGGAUUUACCCGGCAG	GkGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063050	GGGAUUUACCCGGCAG	GmGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063071	GGGAUUUACCCGGCAG	UUGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063001	GGGAUUUACCCGGCAG	UUGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063043	GGGAUUUACCCGGCAG	AAGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC	UCGGGCGUCCUGGAUCUGC	(SEQ ID NO:__)
2002063005	GGGAUUUACCCGGCAG	CAGCUACG	AUCCAGCUGAUGAGUCCCAAAUAGGACGAAACG		(SEQ ID NO:__)

NNGCUACG ------- CAAAUA

B

Seq.	Freq. (%)
GGGCUACG	36
CGGCUACG	15
UGGCUACG	12
AGGCUACG	12
GCGCUACG	6
GAGCUACG	3
GUGCUACG	3
UUGCUACG	6
AAGCUACG	3
CAGCUACG	3

Figure 38

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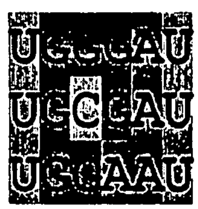
	GGGACUUAAGCCCACUGAUGAGUCGC	nnnnnn	GCGACGAAACGCCUUCGGGCGUCUGGGCAGUCCC	(SEQ ID NO:__)
2002063054	GGGACUUAAGCCCACUGAUGAGUCGC	UGGGAU	GCGACGAAACGCCUUCGGGCGUCUGGGCAGUCC	(SEQ ID NO:__)
2002063034	GGGACUUAAGCCCACUGAUGAGUCGC	UGGGAU	GCGACGAAACGCCUUCGGGCAUCACGAAUUC	(SEQ ID NO:__)
2002063033	UGGACUUAAGCCCACUGAUGAGUCGC	UGGGAU	GCGACGAAACGCCUUCGGGCGUCUGGGCAGUCCC	(SEQ ID NO:__)
2002063032	GGGACUUAAGCCCACUGAUGAGUCGC	UGGGAU	GCGACGAAACGCCUUCGGGCG	(SEQ ID NO:__)
2002063029	UGGACUUAAGCCCACUGAUGAGUCGC	UGGGAU	GCGACGAAACGCCUUCGGGCGUCUGGGCAGUCCC	(SEQ ID NO:__)
2002063031	UGGACUUAAGCCCACUGAUGAGUCGC	UGCGAU	GCGACGAAACGCCUUCGGGCGUCUGGGCAGUCCC	(SEQ ID NO:__)
2002063052	UGAUCUUAAGCCCACUGAUGAGUCGC	UGCGAU	GCGACGAAACGCCUUCGGGCGUCUGGGCAGUCCC	(SEQ ID NO:__)
2002063056	GGGACUUAAGCCCACUGAUGAGUCGC	UGWGAU	GCGACGAAACGCCUUCGGGC	(SEQ ID NO:__)
2002063046	GGGACUUAAGCCCACUGAUGAGUCGC	UGGAAU	GCGACGAAACGCCUUCGGAUCACGAAUUC	(SEQ ID NO:__)



50%
30%
10%

Figure 39

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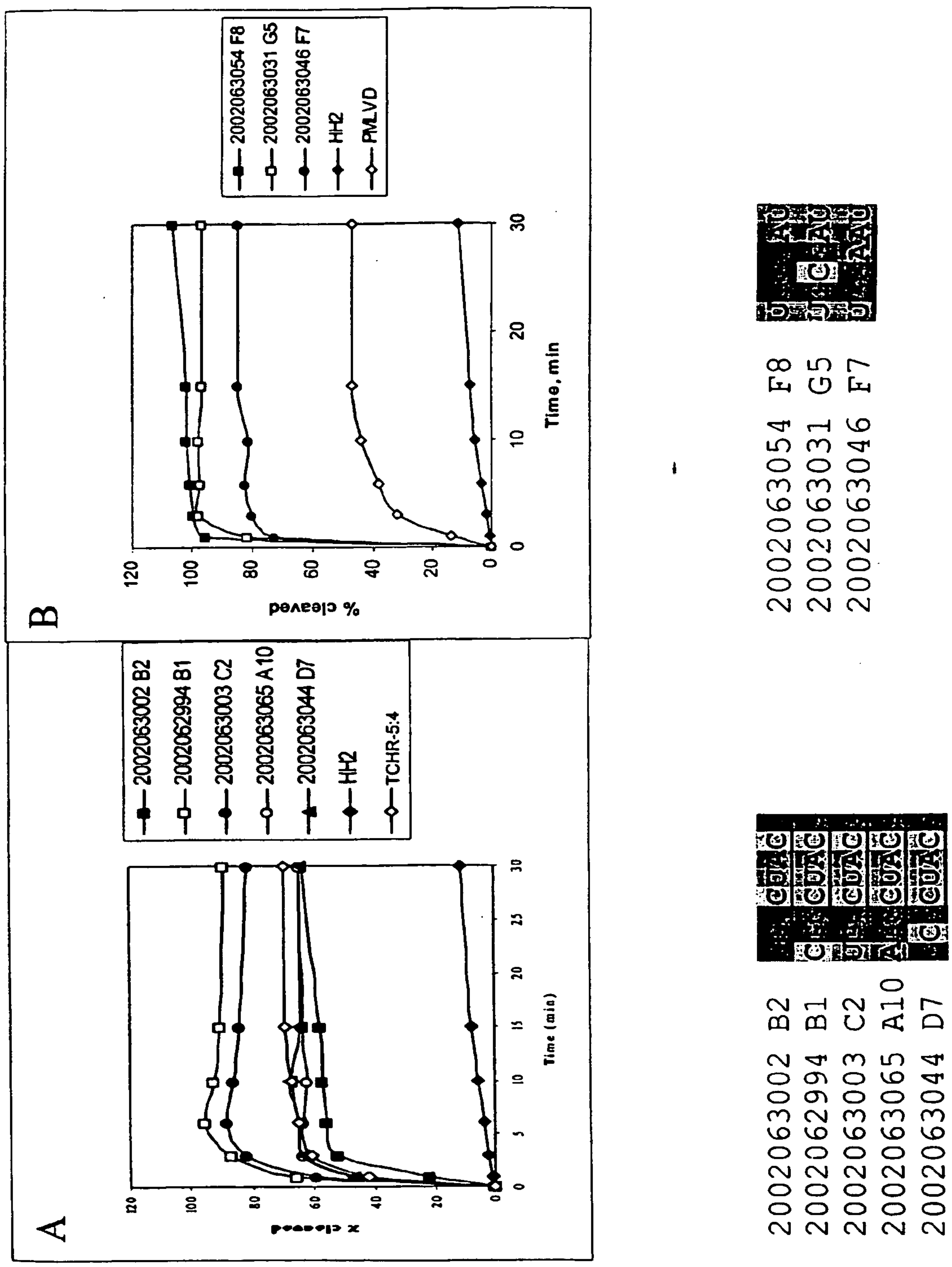

Figure 40

Figure 41A

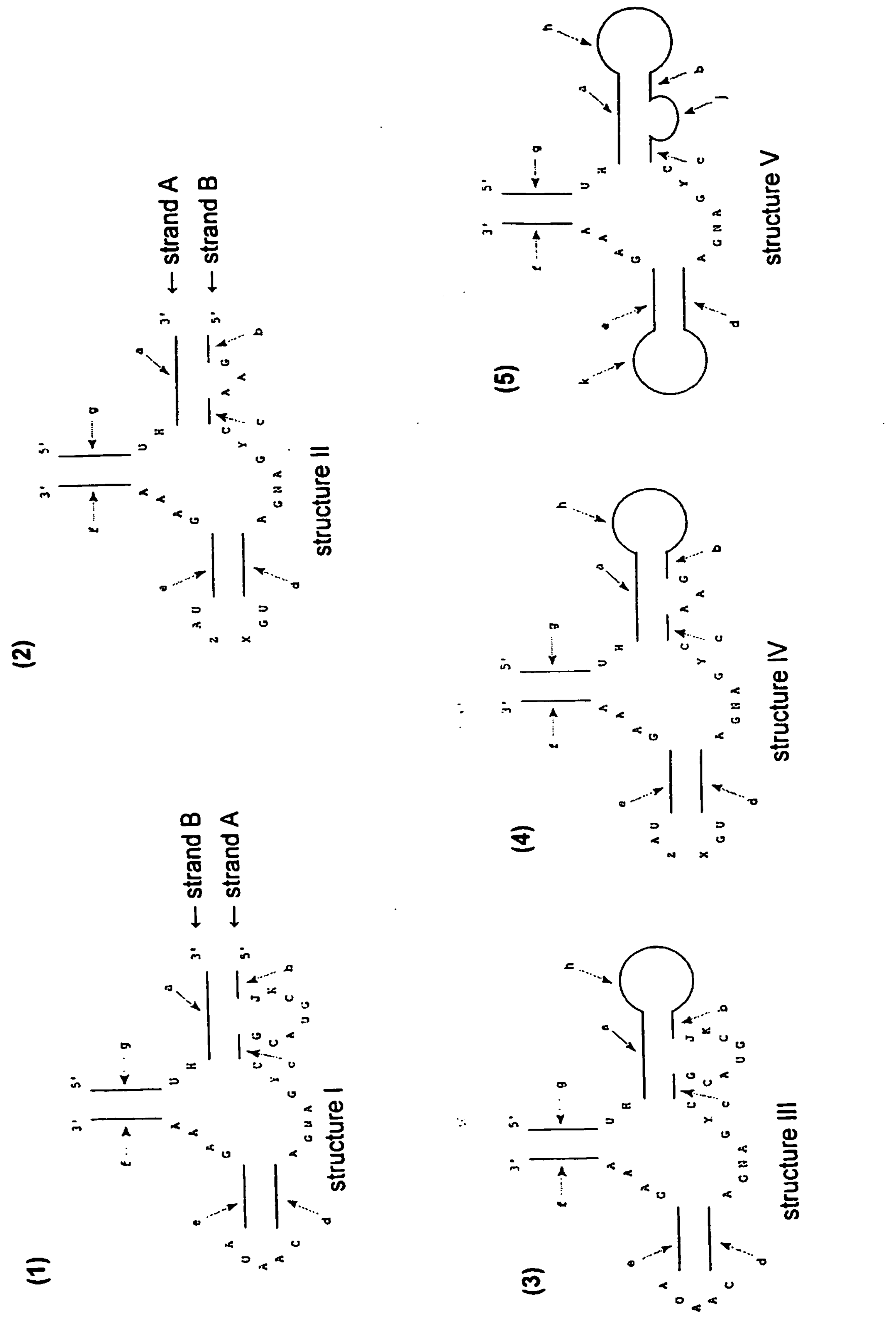
(1)
← strand B
← strand A
structure I
(2)
← strand A
← strand B
structure II
(3)
structure III
(4)
structure IV
(5)
structure V

Figure 41B

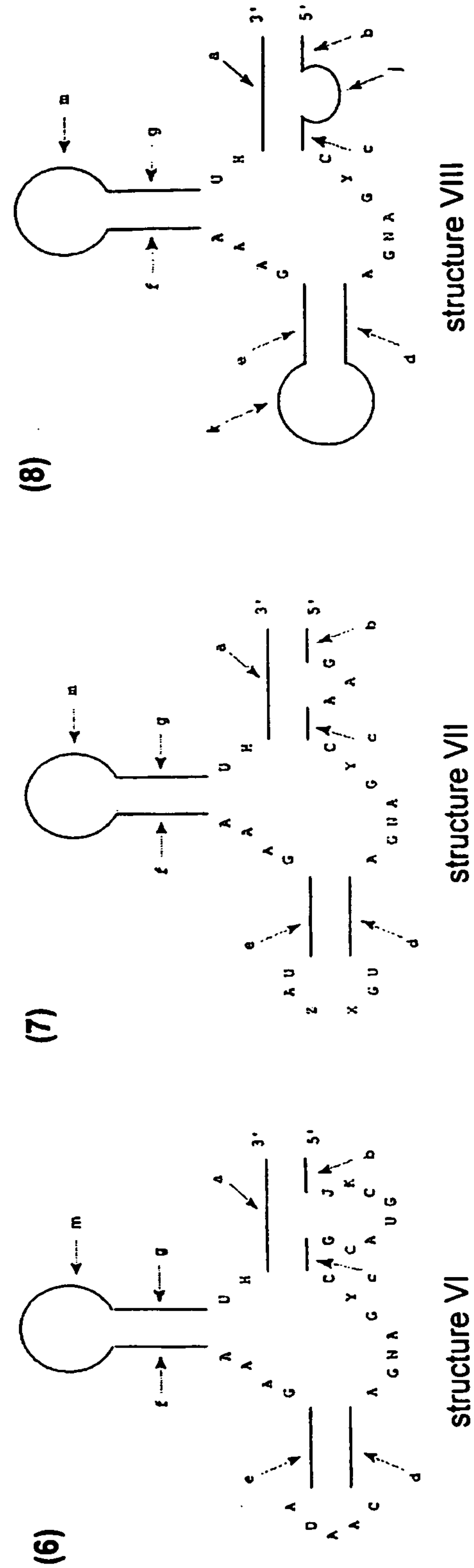

Figure 42A

Notophthalmus viridescens satellite RNA (newt)

```
UUGGAUUCAAGCUAGCCUGG CUGAUGAA GGGUG  A  UACCC AGAAA  CC GG    UC CUAGGAUGCUUUGUUUCCGG
       I'                C      II   LII  II'    C    III III'  CS          I
```

Amb.talpoideum (Am. ta.)

```
GACCCAAGCUACUCCUCA CUGAUGAG GCC CAACAA GGC UGAAA CA  UG   UU UGGGGAUGCUUGUGGUC
       I'              C     II   LII   II'   C   III III' CS        I
```

Amp.tridactulum (Am. tr.)

```
CUUAAGCUGCACCUCA CUGAUGAU GCC CAAUGA GGC UGAAA CG  CG   UU UGGGGUUGCUUGAG
       I'            C     II   LII   II'   C   III III' CS       I
```

Schistosoma (Schistozyme)

```
GGCAGGUACAUCCAG CUGACGA GUCC CAAAUA GGAC GAAA UGCCU  UC  GGGCA UC CUGGAUUCCACUGCU
       I'          C      II    LII   II'   C    III   LIII  III' CS        I
```

Cricket Pst3 from D.baccettii (D. ba.)

```
GUGUGUUCCCUCUGCCCCG CUGAUGA GGUC GGGGA GACC GAAA GGGU   CA   ACUC UA CGGGGCUAUUACAUGC
       I'              C     II   LII   II'  C   III    LIII   III' CS       I
```

Cricket from D.schiavazzii (D. sc.)

```
GAUGUGUGUUCCCUCUGCCCCG CUGAUGA GGUC AGGGAA GACC GAAA GGGU  CG  ACUC  UA CGGGGCUAUAACAUGCAAU
          I'              C      II    LII   II'  C   III  LIII  III' CS         I
```

Avocado sunblotch viroid (ASBV+)

```
GGAAAGAUGGGAAGAACA CUGAUGA GUCUCGCAAGGUUUA----UAAACUUUGUUUGAC GAAA CC    A    GG   UC UGUUCCGACUUUCC
        I'             C         II          LII       II'          C    III  LIII  III' CS        I
```

Figure 42B

Avocado sunblotch viroid (ASBV-)

UUCCCAUCUUUCC CUGAAGA GACGAAGUGA----UCACAAGUC GAAA CUC A GAG UC GGAAAGUCGGAA

I' C II LII II' C III LIII III' CS I

Carnation small viroid-like RNA (CarSV+ RNA)

UUCGAGCCUUACCGACA CUGAUGA GCCAAGAGGAACUUGGAGGC----GCCUCCAAGGGGCCUGGAGGC GAAA CCCC GGGG UC UGUUGGGACCACUCGGA

I' C II LII II' C III III' CS I

HH2

GCGAUGAC CUGAUGA GGCC GAAA GGCC GAAA CGUUCUC GCGA GAGAACG UC GUCGUCGC

I' C II LII II' C III LIII III' CS I

Small circular cherry RNA (Scc+)

AUGCUG UA GUGGGA UGUGUG UCUCAC CUGAAGA GGAC AAAA GUCC GAAA CGGUAU

III' CS I LI I' C II LII II' C III

Small circular cherry RNA (Scc-)

GCUA UA UGGGGA UGUGUG UCCCUA CUGACGA GUUC AAAA GAAC GAAA UAGU

III' CS I LI I' C II LII II' C III

Lucerne transient streak virusoid (sLTSV+)

UACG UC UGAGCG UGAUACC CGCUCA CUGAAGAU GGCCC GGUA GGGCC GAAA CGUA

III' CS I LI I' C II LII II' C III

Lucerne transient streak virusoid (sLTSV-)

GACG UA UGAGAC UGACUGAAACGCC GUCUCA CUGAUGA GGCC AUGGCA GGCC GAAA CGUC

III' CS I LI I' C II LII II' C III

Figure 42C

Tobacco ringspot virus satellite RNA (sTRSV+)

CCUG UC ACCGGA UGUGCUU UCCGGU CUGAUGA GUCC GUGA GGAC GAAA CAGG

III' CS I LI I' C II LII II' C III

Arabis mosaic virus (sArMV)

ACUG UC GCCGGAU GUGU AUCCGAC CUGACGAU GGCCC AAAA GGGCC GAAA CAGU

III' CS I LI I' C II LII II' C III

Chicory yellow mottle virus satellite RNA (sCYMV)

UACUG UC GCC AGACGUGGACCC GGC CUGAUGA GUCC GAAA GGAC GAAA CAGUA

III' CS I LI I' C II LII II' C III

Barley yellow dwarf virus satellite RNA (sBYDV-)

GGUG UC UCAAGGU GCGU ACCUUGA CUGAUGA GUCC GAAA GGAC GAAA CACC

III' CS I LI I' C II LII II' C III

Barley yellow dwarf virus satellite RNA (sBYDV+)

GUGGA UA ACAG AGCGCGUA CUGU CUGACGAC GUAUCCGCGCGGACUAGAAGGC UGGU GCCUCGUCCAACAAAUAGAUAC AGAAA UCCAC

III' CS I LI I' C II LII II' C III

Peach latent mosaic (PLMVd +)

GAAGAG UC UGUGC UAA GCACA CUGACGA GUCUC UGAGAU GAGAC GAAA CUCUUC

III' CS I LI I' C II LII II' C III

Peach latent mosaic (PLMVd-)

UCAUAAG UC UGGGC UAA GCCCA CUGAUGA GUCGC UGAAAU GCGAC GAAA CUUAUGA

III' CS I LI I' C II LII II' C III

Figure 42D

Chrysanthemum chlorotic mottle viroids (CChMVd+)

AAGAGG UC GGCACC UGACGUC GGUGUC CUGAUGAA GAUCC AUGACA GGAUC GAAA CCUCUU

III' CS I LI I' C II LII II' C III

Chrysanthemum chlorotic mottle viroids (CChMVd-)

UCCAG UC GAGACCU GAAGU GGGUUUC CUGAUGA GGCUGUGGAGAGAGC GAAA GCUUUACUCCCACACAAGCC GAAA CUGGA

III' CS I LI I' C II LII II' C III

Subterraneum clover mottle virusoid (vSCMoV)

CGCUG UC UGUACU UGUAUC AGUACA CUGACGA GUCC CUAAA GGAC GAAA CAGCG

III' CS I LI I' C II LII II' C III

Velvet tobacco mottle virusoid (vVTMoV)

UCCG UA GUGGAU GUGU AUCCACU CUGAUGA GUCC GAAA GGAC GAAA CGGA

III' CS I LI I' C II LII II' C III

FIGURE 43

A. TEMPLATE SEQUENCES

STOBRV+	TAATACGACTCACTATGGGACCTGTCACCGGATGTGCTTTCCGGTCTGATGAGTCCGTGAGGACGAAACAGGTCCC
VLTSV-A	TAATACGACTCACTATGGGATACGTCTGAGCGTGATACCCGCTCACTGAAGAGGCCCGGTAGGGCCGAAACGTATCCC
PLMVD-	TAATACGACTCACTATGGGATCATAAGTCTGGGCTAAGCCCACTGATGAGTCGCTGAAATGCGACGAAACTTATGATCC
STOBRV+LT1	TAATACGACTCACTATGGGACCTGTCACCGGATGATACCTCCGGTCTGATGAGTCCGTGAGGACGAAACAGGTCCC
STOBRV+LT2	TAATACGACTCACTATGGGACCTGTCACCGGATGTGCTTTCCGGTCTGATGAGTCCCGGTAGGGACGAAACAGGTCCC
STOBRV+LT1&2	TAATACGACTCACTATGGGACCTGTCACCGGATGATACCTCCGGTCTGATGAGTCCCGGTAGGGACGAAACAGGTCCC
STOBRV+PL1	TAATACGACTCACTATGGGACCTGTCACCGGTAACCGGTCTGATGAGTCCGTGAGGACGAAACAGGTCCC
STOBRV+PL2	TAATACGACTCACTATGGGACCTGTCACCGGATGTGCTTTCCGGTCTGATGAGTCCCTGAAATGGGACGAAACAGGTCCC
STOBRV+PL1&2	TAATACGACTCACTATGGGACCTGTCACCGGTAACCGGTCTGATGAGTCCCTGAAATGGGACGAAACAGGTCCC

B. ANTISENSE SEQUENCES

TobRV-antisence	CACGGACTCATCAGACCGGAAAGCAC
LTSV-antisense	ACCGGGCCTCTTCAGTGAGCGGGTATC
PLMVD- antisense	ATTTCAGCGACTCATCAGTGGGCTTA
STOBRV+LT1- antisense	CACGGACTCATCAGACCGGAGGTATC
STOBRV+LT2- antisense	TACCGGGACTCATCAGACCGGAAAGCA
STOBRV+LT1&2- antisense	TACCGGGACTCATCAGACCGGAGGTAT
STOBRV+PL1- antisense	TCACGGACTCATCAGACCGGTTA
STOBRV+PL2- antisense	TTCAGGGACTCATCAGACCGGAAAGCA
STOBRV+PL1&2- antisense	ATTTCAGGGACTCATCAGACCGGTTA